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Faculdade de Odontologia de Araraquara

Elton Carlos Pichotano

Influência da fibrina rica em plaquetas e leucócitos na formação óssea após cirurgia de elevação do assoalho do seio maxilar com osso bovino desproteinizado: estudo clínico randomizado

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Orientadora: Daniela Leal Zandim-Barcelos

Co-orientador: Elcio Marcantonio Júnior

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DADOS CURRICULARES

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Pichotano E C. Influência da fibrina rica em plaquetas e leucócitos na formação óssea após cirurgia de elevação do assoalho do seio maxilar com osso bovino desproteinizado: estudo clínico randomizado [Tese de Doutorado]. Araraquara: Faculdade de Odontologia da UNESP; 2018.

RESUMOO substituto ósseo de origem bovina, conhecido como osso bovino desproteinizado (OBD), tem demonstrado resultados favoráveis e seguros para regeneração óssea nos procedimentos de levantamento de seio maxilar. No entanto, com o intuito de diminuir o tempo de reparo dos enxertos ósseos, diferentes fatores de crescimento têm sido investigados para serem utilizados associadamente aos substitutos ósseos osteocondutores. O objetivo geral deste estudo clínico randomizado foi avaliar o efeito da fibrina rica em plaquetas e leucócitos (L-PRF) na regeneração óssea quando usada em combinação com OBD na região posterior de maxila após procedimentos cirúrgicos de elevação da membrana sinusal. Um total de 24 pacientes com edentulismo bilateral na região posterior da maxila foram incluídos. Foi utilizado o modelo de boca dividida para utilização dos seguintes biomateirias no procedimento de elevação do seio maxilar: Grupo controle – ODB; Grupo teste – L-PRF + ODB. O período de cicatrização para instalação dos implantes foi de 8 meses no Grupo Controle, enquanto no grupo teste metade dos pacientes receberam implantes com 4 meses (Subgrupo 1, n=12) e a outra metade com 8 meses (Subgrupo 2, n=12). Todos os pacientes realizaram tomografia computadorizada no pré-operatório, imediatamente após a cirurgia e no momento da instalação dos implantes para avaliação da estabilidade do volume ósseo obtido com o procedimento regenerativo. No momento da instalação dos implantes foram obtidas biópsias para análise histomorfométrica. A estabilidade primária dos implantes foi mensurada por análise de frequência de ressonância. No subgrupo 1, a redução do volume ósseo após o período de cicatrização foi semelhante para os grupos teste (33,1%) e controle (36,7%) (p=0,093). No entanto, no subgrupo 2 foi observada uma diferença significativa no percentual de redução do volume ósseo entre os grupos (teste 39,1% e controle 26,8%; p=0,0037). Em relação ao percentual de osso neoformado, houve uma diferença estatística significante entre os grupos tanto no subgrupo 1 (teste - 44.58 ± 13.89%, controle - 30.01 ± 8.41%; p=0,0087) como no subgrupo 2 (teste - 46.5 ± 12.27%, controle - 34.51 ± 7.81%; p<0,05). A estabilidade primária dos implantes do grupo controle (75,13 ± 5,68) foi significativamente maior que dos implantes do grupo teste (60,90 ± 9,35) no subgrupo 1 (p=0,0003). No subgrupo 2, não houve diferença na estabilidade primária dos implantes entre os grupos (controle – 74,74 ± 7,73, teste – 71,95 ± 6,31; p0,35). Pode-se concluir que a associação de L-PRF ao OBD induziu nova formação óssea e reduziu o tempo de cicatrização para instalação de implantes após procedimento de elevação do seio maxilar. No entanto, foi observado uma redução significativa do volume ósseo quando os implantes foram instalados após 8 meses de cicatrização nos seios enxertados com associação de L-PRF e OBD.

Palavras chave: Regeneração óssea. Seio maxilar. Implantação dentária. Agregação plaquetária.

Pichotano E C. The influence of leukocyte and platelet-rich fibrin on bone formation after maxillary sinus floor elevation with deproteinized bovine bone: a randomized clinical trial [Tese de Doutorado]. Araraquara: Faculdade de Odontologia da UNESP; 2018.

ABSTRACT

The deproteinized bovine bone mineral (DBBM) has been showed favorable and safe results for bone regeneration in maxillary sinus lift procedures. However, in order to decrease the healing time of bone grafts, different growth factors have been investigated to be used in association with osteoconductive bone substitutes. The general objective of this split-mouth randomized clinical trial was to evaluate the effect of leukocyte-platelet rich fibrin (L-PRF) to accelerate bone regeneration when used in combination with DBBM in maxillary sinus lift procedures. A total of 24 patients with bilateral edentulism in the posterior region of the maxilla were included. The split-mouth model was used to perform the following treatments for maxillary sinus elevation. Control group – DBBM; Test Group - L-PRF + DBBM. In both groups, a bovine collagen membrane was placed on the window of access to the maxillary sinus. The healing period for implant installation was 8 months in the Control Group, while in the test group half of the patients received implants with 4 months (Subgroup1, n=12) and the other half with 8 months (Subgroup 2, n=12). Tomography's of all the patients were captured in the preoperative period, immediately after the surgery and at the moment of implants placement in order to evaluate the stability of the bone volume obtained with the regenerative procedure. At the time of implants placement, biopsies were obtained for histomorphometric analysis. The primary implant stability was measured by a resonance frequency analysis. In subgroup 1, the bone volume reduction after healing period was similar for the test (33.1%) and control groups (36.7%) ($p = 0.093$). However, in subgroup 2, a significant difference was observed in the percentage of bone volume reduction between groups (test 39.1% and control 26.8%, $p = 0.0037$). In relation to newly formed bone percentage, statistically significant difference was observed between subgroup 1 (test - 44.58 ± 13.89%, control - 30.01 ± 8.41%, $p = 0.0087$) and subgroup 2 (test - 46.5 ± 12.27%, control - 34.51 ± 7.81%, $p <0.05$). The primary implant stability for the control group (75.13 ± 5.68) was significantly higher than for test group (60.90 ± 9.35) in subgroup 1 ($p = 0.0003$). In subgroup 2, there was no difference in the primary implant stability between groups (control - 74.74 ± 7.73, test - 71.95 ± 6.31; p0.35). The L-PRF and DBBM association induced new bone formation and reduced the healing period for implant placement after maxillary sinus lift procedure. However, a significant bone volume reduction was observed when the implants were placed after 8 months of healing in L-PRF and DBBM sites.

Keywords: Bone regeneration. Maxillary sinus. Dental implantation. Platelet aggregation.

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1 INTRODUÇÃO

A qualidade e o volume ósseo adequado são fatores fundamentais para a reabilitação protética posterior de maxilas atróficas. O processo de reabsorção alveolar, inevitável pós-extração dentária, associado à pneumatização da cavidade do seio maxilar com extensão alveolar impede, muitas vezes, a instalação de implantes osseointegráveis com finalidade protética nas regiões posteriores da maxila. Técnicas de enxertia óssea propostas pela literatura mostram a possibilidade do restabelecimento em espessura e altura de rebordos atrésicos com finalidade reabilitadora[1-4]. Dentre as opções de tratamento que têm sido propostas para a reabilitação da região posterior de maxilas atróficas destacam-se a instalação de implantes curtos[5], a técnica de implante batido[6], o enxerto ósseo em bloco sobre a cortical óssea alveolar[7] e a diminuição da cavidade do seio maxilar com enxerto ósseo particulado[8]. A técnica modificada por Boyne e James[8] e desenvolvida por Tatum[9] para preenchimento ósseo do seio maxilar com acesso lateral por meio de uma janela tem sido a forma mais segura e comumente utilizada para aumento ósseo nas região posterior de maxila atrófica para reabilitação com implantes. A técnica é bem documentada cientificamente, entretanto não há o consenso em relação ao biomaterial ideal que deve ser utilizado para o preenchimento da cavidade do seio maxilar.

É evidente na literatura a eficácia do uso de diferentes biomateriais no interior do seio maxilar[10-16]. Entretanto, as pesquisas vêm sendo desenvolvidas para melhorar a eficiência e acelerar a maturação do enxerto ósseo alveolar. O intuito é diminuir o tempo de cicatrização do enxerto com utilização dos fatores de crescimento. Os fatores de crescimento são proteínas bioativas que controlam o processo de cicatrização da ferida, pois têm papel crucial na migração celular, proliferação celular e angiogênese para regeneração dos tecidos[17]. Estes fatores de crescimento podem ser encontrados no sangue, especificamente nas plaquetas e no plasma.

Concentrados de plaquetas, tais como plasma rico em plaquetas (PRP), fibrina rica em plaquetas (PRF) e fator de crescimento concentrado (CGF) têm sido utilizados para a reconstrução de defeitos ósseos[18]. Concentrados autógenos de plaquetas foram inicialmente utilizados no tratamento e prevenção de hemorragias a partir de trombocitopenias graves, muitas vezes causada por aplasia medular,

leucemia aguda ou perda significativa de sangue durante uma cirurgia de longa duração. O concentrado de plaquetas mais utilizado nesses casos tem sido chamado de plasma rico em plaquetas (PRP), contendo em média 0,5 x 10^{11} plaquetas por unidade.

Infelizmente, os primeiros resultados envolvendo a utilização do PRP indicam que o potencial efeito das citocinas, liberadas durante a ativação plaquetária e a coagulação de fibrina, parece ser extremamente limitado ao tempo, sendo liberadas muito rapidamente. O PRP vem sendo utilizado em conjunto com diversos materiais de enxerto em procedimentos de aumento ósseo desde a data da sua introdução. No entanto, os resultados são bastante controversos e ainda não é possível tirar conclusões definitivas sobre o efeito do PRP na regeneração óssea[19-22].

A utilização de derivados do sangue para o selamento de feridas começou com a utilização de colas de fibrina, descritos inicialmente há mais de 40 anos, que eram constituídas de fibrinogênio concentrado[23] (polimerização induzida por trombina e cálcio). Apesar do gel de fibrina oferecer um perfeito suporte para a ação das citocinas plaquetárias, estas pequenas moléculas solúveis são liberadas muito rapidamente com a finalidade de incorporar a matriz de fibrina durante sua polimerização. Além disso, a utilização de trombina bovina e cloreto de cálcio para induzir a polimerização da fibrina influencia consideravelmente as características mecânicas e biológicas da matriz de fibrina[24,25]. O uso de adesivos de fibrina está bem documentado na literatura[26], porém permanece controversa a sua utilização devido à complexidade dos protocolos de produção (para adesivos autólogos) ou risco de infecção cruzada (para adesivos comerciais). Foi então que a nova família de agregados plaquetários foi desenvolvida na França, chamada de fibrina rica em plaquetas (PRF) ou fibrina rica em plaquetas e leucócitos (L-PRF), que se parece com uma matriz cicatricial autógena, rica em plaquetas e leucócitos[27]. Normalmente a literatura associa o termo L-PRF ao nome do autor responsável pela descoberta Choukroun.

A fibrina rica em plaquetas e leucócitos (L-PRF) se apresenta como uma membrana gelatinosa constituída de uma concentração de plaquetas e leucócitos favoráveis à cicatrização e imunidade[28]. A L-PRF influencia diretamente nos fenômenos e complicações de remodelação dos tecidos, melhorando os resultados e diminuindo os riscos de problemas infecciosos e hemorrágicos[29].

A membrana de L-PRF consiste em uma malha de fibrina de alta densidade e com junções trimoleculares equilaterais entre si resultante de uma polimerização lenta com a incorporação de plaquetas, leucócitos e fatores de crescimento[30].

A eficiência da L-PRF reside na aplicação localizada e contínua de uma vasta gama de fatores de crescimento e proteínas, suprindo as necessidades nos processos de cicatrização de feridas e nos processos fisiológicos de reparação tecidual[31]. As suas vantagens mais conhecidas sobre o PRP incluem a facilidade de preparação/aplicação, custo mínimo, e a ausência de modificação bioquímica[32] (sem utilização de trombina bovina ou anticoagulante). Assim que o tecido sanguíneo é coletado e entra em contato com o tubo de ensaio, o processo de coagulação é iniciado, por isso o manuseio deve ser feito o mais rápido possível. Isso pode diminuir o tempo de centrifugação para obtenção da L-PRF. O processo de centrifugação ativa o processo de coagulação sanguínea resultando em um coágulo composto por uma rede de fibrina na qual as plaquetas e outras células do sangue são aprisionadas. Há uma liberação gradual e rápida de fatores de crescimento na rede de fibrina. São liberadas fator de crescimento derivado de plaquetas (PDGF), fator de crescimento endotelial vascular (VEGF), fator de crescimento transformador - beta 1 (TGF-b) e trombospondina em 7-14 dias, precisamente quando a angiogênese tem um pico e inicia-se o crescimento ósseo[33].

Menores quantidades de trombina presentes na L-PRF implicam em uma polimerização da matriz de fibrina formando uma malha mais fina e flexível, o que vai ser absolutamente favorável no suporte ao emaranhado de citocinas plaquetárias e a migração celular. O sucesso da utilização da L-PRF também depende diretamente da velocidade de coleta do sangue e de sua transferência para centrifugação, que teoricamente faz com que as plaquetas fiquem retidas na malha de fibrina formada após a centrifugação. Além disso, a L-PRF não se dissolve rapidamente após a aplicação como acontece com o PRP. A matriz de fibrina é lentamente remodelada de forma semelhante ao coágulo sanguíneo natural, favorecendo o processo de cicatrização[24,30].

Enquanto fatores de crescimento plaquetários estimulam as células do tecido em cicatrização a fim de atraí-los para a regeneração dos tecidos, fibrinas plaquetárias e circulantes começam a formar uma matriz cicatricial densa, buscando fechar a ferida com uma barreira biológica de proteção. A fibrina é a forma ativada de uma molécula plasmática chamada fibrinogênio. Esta molécula fibrilar solúvel

está maciçamente presente tanto no plasma quanto nas plaquetas e desempenham um papel decisivo na hemostasia[17,25,34].

A cicatrização de feridas é completamente dependente dos mecanismos iniciais da hemostasia. Quando um organismo é traumatizado, o primeiro tecido a reagir é o sangue. A ferida desencadeia uma cascata de reações que levam à vedação da violação vascular com plaquetas agregadas[25]. Durante a produção da L-PRF, outros elementos celulares, como os leucócitos, são ativados em adição às plaquetas. As plaquetas levam ao local da ferida uma carga maciça de fibrinogênio e enzimas, e também libera diferentes moléculas em alta quantidade, particularmente fatores de crescimento. Após o fenômeno hemostático e inflamatório artificial induzido pela centrifugação, as plaquetas liberam citocinas pró-inflamatórias interleucina-1 beta (IL-1β), interleucina-6 (IL-6) e fator de necrose tumoral alfa (TNF-a), citocinas anti-inflamatórias interleucina-4 (IL-4) e um promotor de angiogênese[17,34,35] (VEGF).

A L-PRF tem sido usada como único material de preenchimento ou associado aos materiais de enxerto de diferentes caraterísticas com a finalidade de alcançar os melhores resultados nos procedimentos regenerativos[29,36-40].

Os estudos iniciais em animais feitos por Kim et al.[36] combinaram PRF com enxerto de osso medular bovino para regeneração óssea de defeitos criados em calota craniana de coelhos. Observou-se uma redução do tempo necessário para a maturação óssea e uma quantidade maior de osso formado após 5 e 6 meses. Esse resultado deve estar relacionado ao fato de que o gel de fibrina é rico em plaquetas e, por sua vez, libera fatores de crescimento capazes de promover a cicatrização de feridas. Thorn et al.[41] relataram que a PRF apresenta uma concentração de fibrinogênio cerca de 12 vezes maior e uma concentração dos fatores de crescimento cerca de oito vezes maior em relação ao PRP.

O potencial da PRF de Choukroun para regeneração óssea foi testado por Mazor et al.[37] em 2009. Implantes foram instalados juntamente com a elevação da membrana do seio maxilar, sendo a cavidade preenchida somente com PRF. Foram operados 25 seios maxilares em 20 pacientes e, após 6 meses, os mesmos foram avaliados por exame radiográfico volumétrico. Foi verificado um ganho ósseo significante entre 7 e 13 mm além do osso residual. Biópsias obtidas de 9 pacientes mostraram um osso vital bem organizado. Assim, a PRF de Choukroun foi considerada por estes autores uma técnica simples e barata que pode ser utilizada

no interior da cavidade do seio maxilar como biomaterial para regeneração óssea ao redor de implantes.

Tatullo et al.[35] acompanharam clinicamente e histologicamente 60 pacientes que necessitavam de enxerto ósseo na cavidade do seio maxilar previamente à instalação dos implantes. O grupo experimental recebeu PRF associada ao enxerto ósseo de origem bovina desproteinizado (Bio-Oss), enquanto o grupo controle recebeu apenas o biomaterial osteocondutor. Foram feitas avaliações histológicas e histomorfométricas com 106, 120 e 150 dias após a cirurgia. No protocolo de 106 dias foram obtidos os dados mais interessantes da eficácia do L-PRF utilizado como material de enxerto. Observou-se a presença de matriz óssea densa ricamente vascularizada misturada com fragmentos de osso lamelar. Os sítios foram capazes de receber implantes após o período de cicatrização de 106 dias e acompanhados sem problemas ou complicações por 36 meses.

Zhang et al.[40] compararam o efeito da PRF combinada com enxerto ósseo de origem bovina e somente o material de enxerto bovino para preenchimento da cavidade do seio maxilar. Foram feitas avaliações clínicas, radiográficas e histológicas após 6 meses da cirurgia inicial. Não foram observadas complicações durante esse período. Exames tomográficos foram realizados imediatamente após a cirurgia, 3 e 6 meses após a cirurgia. Foi observada a presença de tecido mineralizado sem sinais de reabsorção. A porcentagem de osso formado no grupo que utilizou PRF foi superior ao grupo controle. Apesar de não ter sido encontrada diferença estatística na análise histológica, os autores observaram que a presença do material de enxerto no grupo com PRF foi cerca de 1,5 vezes menor do que a presença no grupo controle. Este resultado poderia ser explicado pela liberação de fatores de crescimento que ocorre quando se utiliza a PRF.

Kim et al.[39] compararam a utilização de tricálcio fosfato (TCP), PRF com TCP e proteína óssea morfogenética 2 recombinante humana (rhBMP-2) com TCP na regeneração óssea em cirurgias de elevação da membrana do seio maxilar em coelhos. Amostras histológicas foram obtidas com 3 dias, 1 semana, 2, 4, 6 e 8 semanas após a cirurgia de enxerto. Não foi verificada formação óssea nas análises realizadas com 3 dias. Em todos os períodos observados, o grupo com TCP foi o que apresentou menor formação óssea. A área óssea neoformada foi estatisticamente superior no grupo TCP + PRF do que no grupo de TCP + rhBMP-2.

Em um estudo também em animais, Bolukbasi et al.[42] compararam o uso de PRF associado ou não ao enxerto de compostos bifásico (beta tricálcio fosfato)

particulado na regeneração de defeitos ósseos criados sob tíbias de coelhos. Após o período de 40 dias, observou-se que o osso neoformado apresentava maior maturação óssea quando a associação dos dois materiais foi utilizada. Os autores destacaram ainda que a efetividade do PRF depende não somente das características, mas sim das propriedades do material a ser administrado em conjunto.

Nizam et al[43] compararam o efeito da L-PRF associada ou não à osso bovino desproteinizado (OBD) na regeneração óssea em cirurgias para elevação dos seios maxilares após 6 meses de cicatrização. Neste estudo foi utilizado o modelo de boca dividida para elevação bilateral do seio maxilar em 13 pacientes. Não foi observada diferença qualitativa nas análises histológicas entre os grupos teste e controle. As porcentagens de osso neoformado, de enxerto ósseo residual e de enxerto em contato com osso neoformado foi semelhante entre os grupos. Assim, os dados deste estudo mostraram que a adição de L-PRF não resultou em aumento de osso neoformado após seis meses de cicatrização.

Em uma revisão sistemática recente sobre o potencial regenerativo da L-PRF[44], foi verificado que este concentrado pode ter um efeito positivo na regeneração óssea e na osseointegração. Porém, a evidência científica ainda não é forte para confirmar este efeito. Assim, o nosso objetivo foi realizar um estudo clínico randomizado, controlado, para avaliar o potencial da L-PRF na regeneração óssea quando usada em combinação com Bio-Oss® (Bio-Oss, Geistlich Pharma AG, Wolhusen, Suiça) na região posterior de maxila após procedimentos cirúrgicos de elevação da membrana do seio maxilar. Como a redução do tempo de incorporação do biomaterial traz benefício do ponto de vista clínico para os pacientes que necessitam de levantamento de seio maxilar para instalação de implantes osseointegráveis, nos propusemos a avaliar o efeito adicional da L-PRF em um período precoce e outro tardio de cicatrização

2 PROPOSIÇÃO

Avaliar o efeito da L-PRF em combinação com OBD na neoformação óssea em procedimentos cirúrgicos de elevação da membrana do seio maxilar por meio de análises clínica, histológica e radiográfica.

Publicação 1 Influence of leukocyte and platelet-rich fibrin on volumetric dimensional changes after 8 months of maxillary sinus augmentation with deproteinized bovine bone mineral: a randomized-controlled clinical study.
O objetivo deste estudo foi investigar a influência da L-PRF nas mudaças volumétricas e na estabilidade dos implantes quando associado ao osso bovino desproteinizado em cirurgias de levantamento de seio maxilar após 8 meses de cicatrização.

Publicação 2 Maxillary sinus augmentation with leukocyte and platelet-rich fibrin (L-PRF) and deproteinized bovine bone mineral for bone regeneration improvement at the implant site. A double blinded, randomized-controlled, prospective, and split-mouth clinical trial.
O objetivo desse estudo foi avaliar histologicamente os efeitos da L-PRF na regeneração óssea quando utilizada em combinação com osso bovino desproteinizado no procedimento de elevaçao do seio maxilar.

Publicação 3 Assessment of the efficiency of leukocyte and platelet-rich fibrina (L-PRF) combined with deproteinized bovine bone graft for early implant placement after maxillary sinus augmentation. A double blinded, randomized-controlled, prospective, and plit-mouth clinical trial.
O objetivo desse estudo foi avaliar a eficiência da adição da L-PRF ao osso bovino desproteinizado para colocação precoce de implante após procedimento de elevação do seio maxilar.

Publicação 4 Early placement of dental implants in maxillary sinus grafted with a combination of leukocyte and platelet-rich fibrin (L-PRF) and deproteinized bovine bone mineral.

O objetivo desse estudo foi descrever um caso clínico em que foi realizada instalação precoce de implantes após realização da elevação do seio maxilar com L-PRF e osso bovino desproteinizado.

3 PUBLICAÇÕES

Os resultados desta tese são apresentados em 3 artigos inéditos e 1 artigo com relato de caso clínico completo, informados a seguir.

3.1 Publicação 1

Influence of leukocyte and platelet-rich fibrin on volumetric dimensional changes after 8 months of maxillary sinus augmentation with deproteinized bovine bone mineral: a randomized-controlled clinical study*

Running title: Association of L-PRF and DBBM for sinus augmentation

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[1] Artigo submetido à Clinical Oral Implants Research (ANEXO C)

Abstract

Objectives: This study investigated the influence of leukocyte and platelet-rich fibrin (L-PRF) on volumetric changes and implant stability when added to deproteinized bovine bone (DBBM) after maxillary sinus augmentation..

Materials and methods: Twelve totally or partially edentulous participants in need of bilateral maxillary sinus augmentation were included in this randomized and controlled trial. A split-mouth design was employed, in which the maxillary sinuses were grafted with a mixture of L-PRF and DBBM (test group) or DBBM only (control group). A collagen membrane was used to cover the lateral window after sinus augmentation. Cone beam computed tomography (CBCT) was obtained one week after sinus augmentation and after 8 months of healing. Bone volumetric changes were measured on CBCT scans through the differential hyperdensity color of the images using a specific software. After the healing period, 38 implants were installed and the implant stability quotient (ISQ) was recorded immediately after implant placement using resonance frequency analysis (RFA).

Results: A statistically significant increase in the graft resorption rate for the test group (39.12% ± 16.87) was observed compared to the control group (26.79% ± 12.42) (P<0.05). No statistically significant difference was found between the control (74.74 [77] ± 7.73, mean [median] ± SD) and test (71.95 [74] ± 6.31) groups for ISQ (P=0.35).

Conclusions: The association of L-PRF and DBBM resulted in a significant increase in graft volumetric reduction compared to DBBM alone after 8 months of healing. However, there were no differences in the primary implant stability between groups.

KEYWORDS: Bone substitutes, cone beam computed tomography, implants, maxillary sinus; sinus augmentation; titanium

INTRODUCTION

Rehabilitation of the posterior maxilla with dental implants has been a common procedure in dental practice for many decades. However, this region often shows poor quality bone that could compromise the primary implant stability (Jensen et al., 1996). One alternative to overcome this limitation is the application of longer size implants, however, insufficient bone volume is usually found in the edentulous posterior maxilla with no substantial support for longer sized implants. Moreover, the bone volume available for implant placement in this region is limited by the presence of the maxillary sinus, which can be pneumatized, compounded by loss of alveolar bone height (McAllister, Margolin, Cogan, Taylor & Wollins, 1998).

The sinus augmentation technique was initially proposed by Boyne and James (Boyne & James, 1980) and it has since become the treatment of choice for implant placement in the posterior maxillary implant sites demonstrating severe bone ridge resorption and/or pneumatization of the sinus (Wallace & Froum, 2003). Autogenous bone was initially used to graft the sinus cavity following Schneiderian membrane elevation because of its osteogenic, osteoinductive and osteoconductive potential (Boyne & James, 1980; Del Fabbro, Testori, Francetti & Weinstein, 2004; Hallman, Sennerby & Lundgren, 2002; Hallman, Lundgren & Sennerby, 2001; Hallman, Hedin, Sennerby & Lundgren, 2002). However, the morbidity associated with bone harvesting at the donor site, the limited amount of tissue that can be harvested and the excessive resorption at the recipient site after a long period of healing (Browaeys, Bouvry & De Bruyn, 2007; Clavero & Lundgren, 2003; de Molon et al., 2015; Pjetursson, Tan, Zwahlen & Lang, 2008) inspired more recent investigations to explore alternative graft biomaterials.

Deproteinized bovine bone mineral (DBBM) has been extensively used for sinus augmentation (Rosen, Hobbs & Spector, 2002; Wallace et al., 2005; Carmagnola, Adriaens & Berglundh, 2005). It is a biocompatible and osteoconductive biomaterial that provides an ideal scaffold for new bone formation with high capacity of receiving implants and loading

over time (Chiapasco, Casentini & Zaniboni, 2009; Dos Santos et al., 2016; Wallace & Froum, 2003). Nevertheless, the lack of osteoinductive properties encouraged different research groups to use growth factors in combination with DBBM in order to the reduce the healing time and improve the amount and quality of bone formation (Dohan et al., 2006; Tatullo et al., 2012).

Platelet-rich fibrin (L-PRF) is an autologous fibrin matrix obtained by centrifugation of whole blood in a tube without anticoagulant that is rich in platelets, leucocytes and growth factors (Dohan et al., 2006). Contrary to other platelet concentrates, the fibrin matrix architecture of L-PRF supports the slow release of grown factors during more than 7 days (Dohan Ehrenfest et al., 2012; Dohan Ehrenfest, Del Corso, Inchingolo, Sammartino & Charrier, 2010). Other advantages of L-PRF include the low cost, acessibility and easy process of preparation (Dohan et al., 2006). The L-PRF has been used alone or in combination with bone graft materials for maxillary sinus augmentation. In a recent systematic review (Ali, Bakry & Abd-Elhakam, 2015), it was verified that the use of L-PRF alone as a graft material had promising results for sinus augmentation with simultaneous implant placement. In addition, L-PRF appeared to accelerate maturation of demineralized freeze-dried bone allograft, but had no additional effect in combination with DBBM.

The most common outcomes evaluated in previous studies that used L-PRF for sinus augmentation are based on histologic and histomorphometric analysis, radiographic bone height and implant survival. Tajima et al. (Tajima, Ohba, Sawase & Asahina, 2013) verified the bone volume obtained after six months of sinus augmentation; however, this volume was not compared with the volume obtained immediately after the surgery in order to determine the volumetric stability of the bone graft material. Therefore, the primary aim of this prospective, randomized controlled split-mouth study was to evaluate the influence of L-PRF on volumetric bone changes after sinus augmentation with DBBM through analysis of cone beam computed tomography (CBCT) performed immediately and 8 months after the surgical

procedure. The secondary aim was to compare the primary stability of implants placed in a site previously treated with a sinus augmentation procedure with DBBM or L-PRF + DBBM by means of resonance frequency analysis (RFA). The null hypothesis of present study was that maxillary sinus augmented with DBBM or L-PRF + DBBM would exhibit similar volumetric bone changes after 8 months of healing.

MATERIALS AND METHODS

Study design

This study was designed as a prospective, double-blinded, randomized and controlled split-mouth clinical trial. Totally or partially edentulous participants in need of bilateral maxillary sinus augmentation prior to implant treatment were recruited at the Department of Diagnosis and Oral Surgery of the School of Dentistry at Araraquara – UNESP from May 2015 through August 2016. Only adult patients (20-80years of age) with a residual alveolar bone height of 4 mm or less at the posterior maxilla (based on CBCT) were included in this study. The exclusion criteria were as follows: compromised general health condition, uncontrolled diabetes, smokers or ex-smokers, alcohol and drug abusers, radiotherapy in the head and neck area, pregnancy, therapies with bisphosphonates, hemocoagulative disorders, chronic sinusitis and patients suffering from any pathology in the maxillary sinus. All participants were informed about the treatment and its possible side effects, and signed the written informed consent term. The study protocol was approved by the institutional ethics committee on human research (protocol number 41357514.5.0000.5416) (ANEXO A e B). This clinical trial was conducted in accordance with the Consolidated Standards of Reporting Trials (CONSORT) Statement (Schulz, Altman & Moher, 2010) and the CONSORT flow chart is shown in Figure 1.

L-PRF preparation

L-PRF was prepared according to a previously described protocol (Dohan et al., 2006). Before the surgery procedure, participant`s venous blood sample was taken using 10-mL vacutainers without anticoagulant (BD Vacutainer® Systems - Brazilian Division). The blood was immediately centrifuged at 300 × g (3,000 rpm) for 10 min (Kasvi K14-0815, Curitiba, Brazil). The result of centrifugation process was the formation of a natural fibrin clot at the centre of the tube. Each fibrin clot was removed from the tube and placed in a metal box (Xpression, Intra-lock System, Sao Paulo, Brazil). The fluids present in the fibrin clots were squeezed out to obtain L-PRF membranes.

Sinus augmentation

All participants underwent bilateral sinus augmentation under local anesthesia (2% mepivacaine plus epinephrine 1:100,000; Rio de Janeiro, RJ, Brazil) according to the method described by Tatum (Tatum, 1986). A mucosal crestal incision combined with anterior and posterior releasing vertical incisions allowed the access to the buccal wall of the maxillary sinus after elevation of a mucoperiosteal flap. A bone window was created by osteotomy using a diamond round bur with constant saline irrigation. After careful sinus membrane elevation, the control side was grafted with small particles (0.25-1 mm diameter) of deproteinized inorganic bovine bone (Bio-Oss®, Geistlich Pharma AG, Wolhusen, Switzerland), while the test side was grafted with a mixture of L-PRF membranes and Bio-Oss® (0.25-1 mm diameter). For each membrane of L-PRF (4-5 mL) cut in few fragments, 0.5 g of Bio-Oss® was mixed. The graft materials were gently compacted at the sinus cavity. A resorbable collagen membrane (Bio-Gide®, Geistlich Pharma AG, Wolhusen, Switzerland) was used to cover the lateral window after the graft material was placed. The mucoperiosteal flap was replaced and sutured with non-absorbable nylon 4-0 wire (Ethicon, Johnson & Johnson S.A., Sao Paulo, Brazil)

One single experienced surgeon performed all the surgical procedures. The graft materials were randomly allocated for either side in each participant according to a computer generated randomization list. The numbers were sealed in opaque envelopes by a person not involved in the study and unware of this clinical trial. The surgeon was blinded to the graft material applied to each sinus cavity before graft implantation. After sinus membrane elevation, the envelope containing the treatment indication was opened by the surgeon assistant. The patients were not informed of the assigned materials.

All participants received the same post-operative care regime including antibiotic therapy (500 mg amoxicillin three times daily for 7 days), anti-inflammatory (100 mg nimesulide twice daily), analgesic medication (750 mg paracetamol to be taken as needed every 8 hours), and 0.12% chlorhexidine digluconate mouthwash twice daily for 7 days. The sutures were removed 7 days after surgery and the region remained without direct loading during the bone regeneration phase.

Implant placement

After 8 months of healing, the same surgeon performed the second surgery to install dental implants into both augmented maxillary sinuses. A total of 38 implants (TitamaxTi EX ACQUA, Neodent, Curitiba, Brazil) were placed, 19 in the control side and 19 in the test side, according to the manufacturer`s protocol. All implants had the same diameter (4 mm) and length (11 mm).

Radiographic analysis

Each participant underwent CBCT scanning at 3 time intervals (SCANORA® 3Dx, Soredex, Tuusula, Finland): preoperative (T0), immediately after maxillary sinus augmentation (T1) and 8 months after maxillary sinus augmentation (T2) (Harris et al., 2012). The same parameters were used for all scans, 10 mAs, 90 kVp and a 20 s scan time using the 9 inch

field of view (FOV), with a 350 μm voxel size. The preoperative scan was used to assess the need of sinus augmentation and grafting for implant placement in a two-stage surgical approach. The immediately post-augmentation scan was performed one week after the first surgery. The last scan was performed prior to the second surgery (implant installation). The raw data of the scans were reconstructed and exported in DICOM file format for importing into CBCT interpretation software (Planmeca Romexis 3D module, Planmeca Oy, Helsinki, Finland) as shown in Figure 2

The volumetric measurements (in cubic centimeters) of the grafts was determined using 1 mm sagittal sections through the differential radiodensity of the images, following previously published protocols (Ahlowalia et al., 2013). One maxillofacial radiologist experienced with CBCT that received prior training and blinded to the treatment protocol performed all volumetric measurements in a standardized manner. The mean (SD) graft resorption rate after 8 months of sinus augmentation (i.e. between T1 and T2) was the primary outcome of this study.

The Pearson correlation coefficient (r) was calculated to evaluate the reproducibility of the measurements in the differences of the two measurements performed in five samples before this study. An r-value 0.98 was calculated for the comparison of measurements 1 and 2, indicating near perfect intra-examiner reliability.

Resonance frequency analysis (RFA)

The secondary outcome was the primary implant stability as recorded immediately after implant placement by RFA (Osstell® Mentor device, Osstell AB, Göteborg, Sweden). Smartpegs were attached to the implants and the implant stability quotient (ISQ) measured for each implant (from 1 to 100). The measurements were performed in two directions, buccal-lingual and mesio-distal, and the mean values were used, as previously described (Dos Anjos

et al., 2016). The implant stability was assessed by one examiner who was blinded to the treatment protocol.

Statistical analysis

The statistical analyses were performed with GraphPad Prism software (version 6.0, GraphPad Software, Inc., La Jolla, CA, USA). Data were described using measures of central tendency (mean and median) and of dispersion (standard deviation and 95% confidence interval). The Shapiro-Wilk test was used to assess the normality of data distribution. The bone volume measured immediately after surgery (baseline) for both groups and the ISQ values for the control group did not show normal distribution. The Wilcoxon test was used to assess the changes between time points within each group and to determine any differences between the groups for primary implant stability. Percentage of bone resorption after 8 months of healing between the test and control groups were evaluated by a paired t-test. A statistical significance difference was considered at $p < 0.05$.

RESULTS

Statistical power

G*Power 3.1 (Faul, Erdfelder, Buchner & Lang, 2009) was used to compute achieved statistical power. Considering the mean and standard deviation differences between test and control group for graft resorption rate, an effect size of 2.77 was obtained. Using this effect size, the significance level of 0.05 and the sample size of 12 participants, a statistical power of 100% was obtained.

Participant characteristics

The study consisted of 12 patients, 3 totally and 9 partially edentulous, 9 women and 3 men, with a mean age of 54 years (range 26-69), were enrolled in this clinical trial. Maxillary sinus

augmentation was performed bilaterally in all participants and no complications were observed during or after the sinus augmentation surgery. No perforation of the sinus membrane was verified and no participant was lost to follow-up (Figure 1). The grafts were allowed to integrate for 8 months, when 38 implants were installed, 19 in each group. During the healing period, the participants did not wear any provisional removable denture.

Radiographic analysis

A total of 24 CBCT scans from T1 and T2 of 12 participants were evaluated. The volumetric changes in each grafted sinus are shown in Figures 3 and 4. The mean grafted bone volume one week after surgery (T1) was 1.36 cm^3 (± 0.88, median 1.11, 95% confidence interval 0.80-1.92) for the control group and 1.68 cm^3 (± 1.05, median 1.33, 95% confidence interval 1.01-2.35) for the test group. After 8 months of healing (T2), the mean grafted bone volume was 0.94 cm^3 (± 0.45, median 0.87, 95% confidence interval 0.65-1.23) for the control group and 0.94 cm^3 (± 0.47, median 0.87, 95% confidence interval 0.64-1.25) for the test group. Between T1 and T2 statistically significant reductions in graft volume were noted for both control (P=0.0005) and test (P=0.0005) groups. The mean resorption rate for the control group was statistically significant lower (26.79% ± 12.42, 95% confidence interval 18.90-34.68) in comparison with the test group (39.12% ± 16.87, 95% confidence interval 28.40-49.84) (P=0.0337) (Figure 5).

Resonance analysis

The implants installed in both groups demonstrated high ISQ values indicating good primary stability. No statistically significant differences were found between the control (74.74[77] ± 7.73, mean[median] ± SD) and test (71.95[74] ± 6.31) groups for ISQ (P =0.35) (Figure 6).

DISCUSSION

The present prospective clinical study was designed to determine the tridimensional volume changes after maxillary sinus augmentation with DBBM (Bio-Oss, control group) or L-PRF mixed with DBBM (test group). The volumetric evaluation of the resorption rate of grafted bone was calculated based on two CBCT images, one obtained one week after the sinus augmentation and the other after 8 months of healing. A significant volumetric reduction was observed through time in both groups; however, the sinus augmented with L-PRF and DBBM exhibited higher resorption rates than the control group with only DBBM (P=0.0337). Therefore, the null hypothesis of the present study was rejected.

The edentulous posterior maxilla commonly has insufficient bone volume for implant placement in an optimal position for prosthetic rehabilitation. For this reason, a wide range of bone graft materials have been used for maxillary sinus augmentation. DBBM has been widely used for this purpose (Jensen, Schou, Stavropoulos, Terheyden & Holmstrup, 2012). Previous studies have evaluated the volumetric changes after maxillary sinus augmentation with DBBM through computed tomography resulting in observations of wide variation in resorption rates. Kühl et al. (Kühl et al., 2014) verified in two different groups a significant volume decrease ranging from 15.2% (3% - 27.3%) to 21.5% (11.3% - 37.3%). The loss of volume measured by Kirmeier et al. (Kirmeier et al., 2008) ranged from 0.4 to 33%. Umanjec-Korac et al. (Umanjec-Korac, Wu, Hassan, Liu & Wismeijer, 2014) verified a resorption rate of 19.3% (1.63% - 34.55%) at 2-year follow up in a retrospective study, while Xavier et al. (Xavier et al., 2016) showed a resorption rate of 11.6% (3.17% - 18.47%) for the group grafted with DBBM. On the other hand, Klein et al. (Klein et al., 2016) observed a significant volume increase in all augmented maxillary sinus with mean neoformation of 9.10% (0.33% - 25.97%). In our study, the mean resorption rate of the sinus grafted with DBBM was 26% (6.85% - 50.35%) after 8 months of healing.

Since DBBM does not present osteoinductive properties (Browaeys et al., 2007; Tadjoedin, de Lange, Bronckers, Lyaruu & Burger, 2003), this biomaterial has been used in

combination with bioactive substances presenting growth factors for improving bone regeneration. However, few studies evaluated the influence of this combination on volumetric stability of augmented maxillary sinus. In the present study, the addition of L-PRF resulted in increased bone volumetric reductions: the resorption rate of the sinus grafted with L-PRF and DBBM was 39% (3.59% – 60.38%). This result could be explained by the L-PRF degradation over time (Kobayashi et al. 2016), and the smaller relative amount of DBBM in the test group compared to the control group. Kühl et al. (Kühl et al., 2014) evaluated the influence of bone marrow aspirates (BMA) and concentrates (BMAC) on the volumetric stability of sinus grafted with DBBM. All maxillary sinuses showed significant volume decrease after 6 months of healing. As cited previously, the mean volume reduction for DBBM range from 15.2% (3% - 27.3%) to 21.5% (11.3% - 37.3%), while the mean volume reduction for DBBM + BMA was 20.45% (1.5% - 37.3%) and 16.59% (10.6% - 20.30%) for DBBM + BMAC. The volumetric reduction observed in the test group of our study was lower to the average reduction observed for autogenous bone in particulate or block form in controlled studies (48.04% ranging from 21.5% to 75.1%) on maxillary sinus augmentation (Shanbhag, Shanbhag & Stavropoulos, 2014).

Although the volumetric reductions in augmented maxillary sinuses generally do not compromise implant placement in a two-stage procedure, it is important for the clinician to understand the bone volumetric dimensional changes that can occur after this type of surgery, which in turn influences biomaterial choice and the prognosis of the implant reconstruction (Klein et al., 2016). Long-term stability of the grafted bone volume has been considered an important factor for implant success. An appropriate volume and bone quality of the grafted sinus should be obtained in order to reduce intraosseous stress and tension at the bone-implant interface caused by masticatory forces (Di Lallo et al., 2014; Diserens, Mericske & Mericske-Stern, 2005; Tepper, Haas, Zechner, Krach & Watzek, 2002). Taking into account the findings of the present study, it may be reasonable for clinicians performing maxillary sinus

augmentation using a combination of L-PRF and DBBM to factor in a 40% overestimation of the likely graft volume at the time of implant installation.

CBCT has been considered a reliable technique for 3D visualization of the volumetric bone changes after sinus augmentation. The wide biological variances in volumetric changes of the present study was also observed in previous studies (Kirmeier et al., 2008; Klein et al., 2016; Kühl et al., 2014; Umanjec-Korac et al., 2014). The present study employed a split-mouth design to avoid inter-subject variability, thus increasing the power of the study, and one experienced surgeon performed all surgery procedures. In the present participant cohort, we had a higher percentage of women and the mean age of the patients was 53.92 (±12.76), both may have influenced the resorption rate of the grafting materials. Other possible contributory factors may have included preoperative bone height, compression forces, re-pneumatization forces, and residual wall surface in contact with the grafting material (Gultekin et al., 2016; Kirmeier et al, 2008; Shanbhag et al., 2014; Wallace & Froum, 2003).

The achievement of optimal primary stability has been considered an important factor for implant success (Degidi, Daprile & Piattelli, 2012; Meredith, 1998). After 8 months of healing of the sinus augmentation, 19 implants were placed in each group. The primary stability of these implants was measured by resonance frequency analysis. All implants showed high ISQ values and no significant difference could be observed between the groups. In fact, most of the implants revealed an ISQ value around or higher than 70 in both groups, with a mean of 71.95 and 74.74 for the test and control group respectively. Tajima et al (Tajima et al., 2013) evaluated the primary stability of implants installed in maxillary sinus grafted with only L-PRF after 6 months of healing. The mean ISQ value for seventeen implants was 66.5 (range from 57 to 75 ISQ). Previous studies verified that ISQ values ranging from 55 - 80 represent a safe level of stability, while an ISQ value below 55 should be considered a signal for caution (Sennerby & Meredith, 2008; Nedir, 2004). Sjöström et al. (Sjöström, Sennerby, Nilson & Lundgren, 2007) obtained a low primary stability (ISQ 54.6)

for 17 implants installed in grafted maxilla that failed during the first year of function. Our data suggest that an appropriate maturation of the bone graft was achieved with both treatments, which allow the insertion of implants with an optimal primary stability.

One limitation of this clinical trial is the absence of a group with an early re-entry procedure after sinus augmentation to compare the influence of L-PRF when added to DBBM in the early phase of bone healing process. Moreover, histological analysis could give additional information regarding bone maturation and possible differences between test and control group.

According to our results, the DBBM and the association of L-PRF and DBBM can be used in maxillary sinus augmentation procedure to improve the bone volume necessary to support a dental implant in severe atrophic edentulous posterior maxilla. However, the mixture of L-PRF and DBBM resulted in a significant volumetric reduction compared to DBBM alone after 8 months of healing. These results must be confirmed by larger studies with longer-term evaluation periods.

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Conflict of Interest

The authors have no conflict of interest related to this study.

REFERENCES

Ahlowalia, M. S., Patel, S., Anwar, H. M. S., Cama, G., Austin, R. S., Wilson, R., & Mannocci, F. (2013). Accuracy of CBCT for volumetric measurement of simulated periapical lesions. *International Endodontic Journal,* 46, 538-546. doi: 10.1111/iej.12023

Ali, S., Bakry, S. A., & Abd-Elhakam, H. (2015). Platelet-rich fibrin in maxillary sinus augmentation: A systematic review. *Journal of Oral Implantology,* 41, 746-753. doi: 10.1563/aaid-joi-D-14-00167

Boyne, P. J., & James, R. A. (1980). Grafting of the maxillary sinus floor with autogenous marrow and bone. *Journal of Oral Surgery,* 38, 613-616.

Browaeys, H., Bouvry, P., & De Bruyn, H. (2007). A literature review on biomaterials in sinus augmentation procedures. *Clinical Implant Dentistry and Related Research*, 9, 166–177. doi: 10.1111/j.1708-8208.2007.00050.x

Carmagnola, D., Adriaens, P., & Berglundh, T. (2003). Healing of human extraction sockets filled with Bio-Oss. *Clinical Oral Implants Research*, 14, 137-143.

Chiapasco, M., Casentini, P., & Zaniboni, M. (2009). Bone augmentation procedures in implant dentistry. *International Journal of Oral & Maxillofacial Implants,* 24, Suppl, 237-259.

Choukroun, J., Diss, A., Simonpieri, A., Girard, M. O., Schoeffler, C., Dohan, S. L., Dohan, A. J., Mouhyi, J., & Dohan, D. M. (2006). Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part V: histologic evaluations of PRF effects on bone allograft maturation in sinus lift. *Oral Surgery, Oral Medicine, Oral Pathology Oral Radiology and Endodontology,* 101, 299-303. doi: 10.1016/j.tripleo.2005.07.012

Clavero, J., & Lundgren, S. (2003). Ramus or chin grafts for maxillary sinus inlay and local onlay augmentation: comparison of donor site morbidity and complications. *Clinical Implant Dentistry and Related Research, 5,* 154-160.

Degidi, M., Daprile, G., & Piattelli, A. (2012). Primary stability determination by means of insertion torque and RFA in a sample of 4,135 implants. *Clinical Implant Dentistry and Related Research*, 14*,* 501-507.doi:10.1111/j.1708-8208.2010.00302.x

Del Fabbro, M., Testori, T., Francetti, L., & Weinstein, R. (2004). Systematic review of survival rates for implants placed in the grafted maxillary sinus. *International Jounal of Periodontics Restorative Dentistry,* 24, 565-577.

Di Lallo, S., Ricci, L., Orecchioni, S., Piattelli, A., Iezzi, G., & Perrotti, V. (2014). Resonance frequency analysis assessment of implants placed with a simultaneous or a delayed approach in grafted and nongrafted sinus sites: a 12-month clinical study. *Clinical Implant Dentistry Related Research,* 16, 394-400. doi: 10.1111/cid.12002

Diserens, V., Mericske, E., & Mericske-Stern, R. (2005). Radiographic analysis of the transcrestal sinus floor elevation: short-term observations. *Clinical Implant Dentistry Related Research,* 7, 70-78.

Dohan, D. M., Choukroun, J., Diss, A., Dohan, S. L., Dohan, A. J., Mouhyi, J., & Gogly, B. (2006). Platelet-rich fibrin (PRF): A second-generation platelet concentrate. PartI: technological concepts and evolution. *Oral Surgery, Oral Medicine, Oral Pathology Oral Radiology and Endodontology,* 101, e37-44. doi: 10.1016/j.tripleo.2005.07.008

Dohan Ehrenfest, D. M., Bielecki, T., Jimbo, R., Barbe, G., Del Corso, M., Inchingolo, F., & Sammartino, G. (2012). Do the fibrin architecture and leukocyte content influence the growth factor release of platelet concentrates? An evidence-based answer comparing a pure platelet-

rich plasma (P-PRP) gel and a leukocyte- and platelet-rich fibrin (L-PRF). *Current Pharmaceutical Biotechnology,* 13, 1145-1152.

Dohan Ehrenfest, D. M., Del Corso, M., Inchingolo, F., Sammartino, G., & Charrier, J. B. (2010). Platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) in human cell cultures: growth factor release and contradictory results. *Oral Surgery, Oral Medicine, Oral Pathology Oral Radiology and Endodontology,* 110, 418-421; author reply 421-41. doi: 10.1016/j.tripleo.2010.05.059

Dos Anjos, T. L., de Molon, R. S., Paim, P. R., Marcantonio, E., Marcantonio, E. Jr., & Faeda, R. S. (2016). Implant stability after sinus floor augmentation with deproteinized bovine bone mineral particles of different sizes: a prospective, randomized and controlled split-mouth clinical trial. *International Journal of Oral and Maxillofacial Surgery,* 45, 1556-1563. doi: 10.1016/j.ijom.2016.09.004

Dos Santos, P. L., de Molon, R. S., Queiroz, T. P., Okamoto, R., de Souza Faloni, A. P., Gulinelli, J. L., Luvizuto, E. R., & Garcia, I. R., Jr. (2016). Evaluation of bone substitutes for treatment of peri-implant bone defects: Biomechanical, histological, and immunohistochemical analyses in the rabbit tibia. *Jounal of Periodontal & Implant Science,* 46, 176-196. doi: 10.5051/jpis.2016.46.3.176.

Faul, F., Erdfelder, E., Buchner, A., & Lang, A. G. (2009). Statistical power analyses using G*power 3.1: tests for correlation and regression analyses. *Behavior Research Methods,* 41**,** 1149-1160. doi: 10.3758/BRM.41.4.1149.

Gultekin, B. A., Cansiz, E., Borahan, O., Mangano, C., Kolerman, R., Mijiritsky, E., & Yalcin, S. (2016). Evaluation of volumetric changes of augmented maxillary sinus with different bone grafting biomaterials. *The Jounal of Craniofacial Surgery,* 27, e144-148. doi: 10.1097/SCS.0000000000002393

Hallman, M., Hedin, M., Sennerby, L., & Lundgren, S. (2002). A prospective 1-year clinical and radiographic study of implants placed after maxillary sinus floor augmentation with bovine hydroxyapatite and autogenous bone. *Journal of Oral and Maxillofacial Surgery,* 60, 277-284.

Hallman, M., Lundgren, S., & Sennerby, L. (2001). Histologic analysis of clinical biopsies taken 6 months and 3 years after maxillary sinus floor augmentation with 80% bovine hydroxyapatite and 20% autogenous bone mixed with fibrin glue. *Clinical Implant Dentistry and Related Research,* 3, 87-96.

Hallman, M., Sennerby, L., & Lundgren, S. (2002). A clinical and histologic evaluation of implant integration in the posterior maxilla after sinus floor augmentation with autogenous bone, bovine hydroxyapatite, or a 20:80 mixture. The *International Journal of Oral and Maxillofacial Implants,* 17, 635-643.

Harris, D., Horner, K., Gröndahl, K., Jacobs, R., Helmrot, E., Benic, G.I., Bornstein, M.M., Dawood, A., & Quirynen, M. (2012). E.A.O. guidelines for the use of diagnostic imaging in implant dentistry 2011. A consensus workshop organized by the European Association for Osseointegration at the Medical University of Warsaw. *Clinical Oral Implants Research,* 23, 1243-1253. doi: 10.1111/j.1600-0501.2012.02441.x

Jensen, S. S., Aaboe, M., Pinholt, E. M., Hjorting-Hansen, E., Melsen, F., & Ruyter, I. E. (1996). Tissue reaction and material characteristics of four bone substitutes. *The International Journal of Oral and Maxillofacial Implants,* 11, 55-66.

Jensen, T., Schou, S., Stavropoulos, A., Terheyden, H., & Holmstrup, P. (2012). Maxillary sinus floor augmentation with Bio-Oss or Bio-Oss mixed with autogenous bone as graft: A systematic review. *The International Journal of Oral and Maxillofacial Implants,* 41, 114-120. doi: 10.1016/j.ijom.2011.08.010.

Kirmeier, R., Payer, M., Wehrschuetz, M., Jakse, N., Platzer, S., & Lorenzoni, M. (2008). Evaluation of three-dimensional changes after sinus floor augmentation with different grafting materials. *Clinical Oral Implants Research,* 19, 366-372. doi: 10.1111/j.1600-0501.2007.01487.x

Klein, G. G., Curvello, V. P., Dutra, R. A., Simeao, S. P., Santos, P. L., Gulinelli, J. L., & Filho, H. N. (2016). Bone volume changes after sinus floor augmentation with heterogenous graft. *The International Journal of Oral and Maxillofacial Implants,* 31, 665-671. doi: 10.11607/jomi.3948.

Kobayashi, E., Fluckiger, L., Fujioka-Kobayashi, M., Sawada, K., Sculean, A., Schaller, B., & Miron, R. J. (2016). Comparative release of growth factors from PRP, PRF, and advanced-PRF. *Clinical Oral Investigations,* 20, 2353-2360. doi: 10.1007/s00784-016-1719-1

Kühl, S., Payer, M., Kirmeier, R., Wildburger, A., Wegscheider, W., & Jakse, N. (2014). The influence of bone marrow aspirates and concentrates on the early volume stability of maxillary sinus grafts with deproteinized bovine bone mineral - first results of a RCT. *Clinical Oral Implants Research,* 25, 221-225. doi: 10.1111/clr.12101.

McAllister, B. S., Margolin, M. D., Cogan, A. G., Taylor, M., & Wollins, J. (1998). Residual lateral wall defects following sinus grafting with recombinant human osteogenic protein-1 or Bio-Oss in the chimpanzee. *The International Journal of Periodontics and Restorative Dentistry,* 18, 227-239.

Meredith, N. (1998). Assessment of implant stability as a prognostic determinant. *The International Journal of Prosthodontics,* 11, 491-501.

Nedir, R., Bischof, M., Szmukler-Moncler, S., Bernard, J.P., Samson, J. (2004). Predicting osseointegration by means of implant primary stability. *Clinical Oral Implants Research,* 15, 520-528. doi: 10.1111/j.1600-0501.2004.01059.x

Pjetursson, B. E., Tan, W. C., Zwahlen, M., & Lang, N. P. (2008). A systematic review of the success of sinus floor elevation and survival of implants inserted in combination with sinus floor elevation. *Journal of Clinical Periodontology,* 35, 216-240. doi: 10.1111/j.1600-051X.2008.01272.x.

Rosen, V.B., Hobbs, L.W., & Spector, M. (2002). The ultrastructure of anorganic bovine bone and selected synthetic hyroxyapatites used as bone graft substitute materials. *Biomaterials*, 23, 921-928

Schulz, K. F., Altman, D. G., & Moher, D. (2010). CONSORT 2010 statement: Updated guidelines for reporting parallel group randomised trials. *Journal of Pharmacology and Pharmacotherapeutics,* 1, 100-107. doi: 10.4103/0976-500X.72352

Sennerby, L., & Meredith, N. (2008). Implant stability measurements using resonance frequency analysis: Biological and biomechanical aspects and clinical implications. *Periodontology 2000,* 47, 51-66. doi: 10.1111/j.1600-0757.2008.00267.x.

Shanbhag, S., Shanbhag, V., & Stavropoulos, A. (2014). Volume changes of maxillary sinus augmentations over time: a systematic review. *International Journal of Oral and Maxillofacial Implants,* 29, 881-892.

Sjöström, M., Sennerby, L., Nilson, H., & Lundgren, S. (2007). Reconstruction of the atrophic edentulous maxilla with free iliac crest grafts and implants: a 3-year report of a prospective clinical study. *Clinical Implant Dentistry Related Research,* 9, 46-59. doi: 10.1111/j.1708-8208.2007.00034.x

Tadjoedin, E. S., de Lange, G. L., Bronckers, A. L., Lyaruu, D. M., & Burger, E. H. (2003). Deproteinized cancellous bovine bone (Bio-Oss) as bone substitute for sinus floor elevation. A retrospective, histomorphometrical study of five cases. *Journal of Clinical Periodontology,* 30, 261–270.

Tajima, N., Ohba, S., Sawase, T., & Asahina, I. (2013). Evaluation of sinus floor augmentation with simultaneous implant placement using platelet-rich fibrin as sole grafting material. *The International Journal of Oral and Maxillofacial Implants,* 28, 77-83. doi: 10.11607/jomi.2613.

Tatullo, M., Marrelli, M., Cassetta, M., Pacifici, A., Stefanelli, L. V., Scacco, S., Dipalma, G., Pacifici, L., & Inchingolo, F. (2012). Platelet rich fibrin (P.R.F.) in reconstructive surgery of atrophied maxillary bones: clinical and histological evaluations. *International Journal of Medical Sciences,* 9, 872-880. doi: 10.7150/ijms.5119.

Tatum, H., Jr. (1986). Maxillary and sinus implant reconstructions. *Dental Clinic of North America,* 30, 207-229.

Tepper, G., Haas, R., Zechner, W., Krach, W., & Watzek, G. (2002). Three-dimensional finite element analysis of implant stability in the atrophic posterior maxilla: a mathematical study of the sinus floor augmentation. *Clinical Oral Implants Research,* 13, 657-665.

Umanjec-Korac, S., Wu, G., Hassan, B., Liu, Y., & Wismeijer, D. (2014). A retrospective analysis of the resorption rate of deproteinized bovine bone as maxillary sinus graft material on cone beam computed tomography. *Clinical Oral Implants Research,* 25, 781-785. doi: 10.1111/clr.12174.

Wallace, S. S., & Froum, S. J. (2003). Effect of maxillary sinus augmentation on the survival of endosseous dental implants. A systematic review. *Annals of Periodontology,* 8, 328-343. doi: 10.1902/annals.2003.8.1.328

Wallace, S.S., Froum, S.J., Cho, S.C., Elian, N., Monteiro, D., Kim, B.S., & Tarnow, D.P. (2005). Sinus augmentation utilizing anorganic bovine bone (Bio-Oss) with absorbable and nonabsorbable membranes placed over the lateral window: histomorphometric and clinical analyses. *The International Journal of Periodontics & Restorative Dentintry,* 25, 551-559.

Xavier, S. P., Santos Tde, S., Sehn, F. P., Silva, E. R., Garcez-Filho Jde, A., & Martins-Filho, P. R. (2016). Maxillary sinus grafting with fresh frozen allograft versus bovine bone mineral: A tomographic and histological study. *Journal of Cranio-Maxillo-Facial Surgery,* 44, 708-714. doi: 10.1016/j.jcms.2016.03.005.

FIGURE LEGENDS

Figure 1- CONSORT flowchart of the study showing participant flow through enrollment, allocation, follow-up and analysis. No loss to follow up was observed.

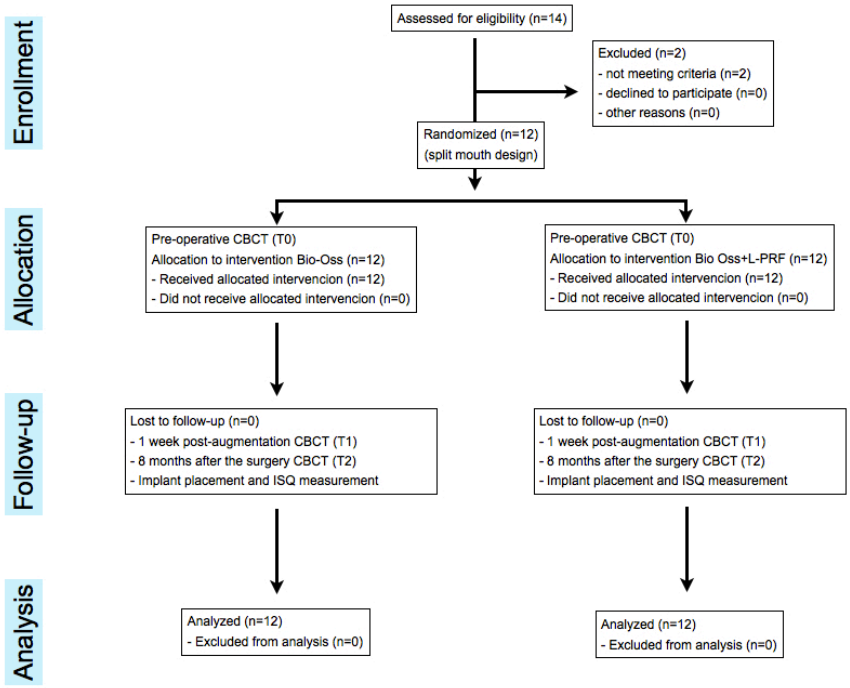


Figure 2- CTBC was performed (a) before sinus augmentation surgery (T0), (b) one week after the surgical grafting procedure (T1), (c) 8 months post-augmentation and before implant placement (T2). (d) Radiographic analysis was performed by selecting the grafted area according to the different gray value using segmentation application within Planmeca Romexis 3D imaging module at both T1 and T2 and calculating the percent change in graft volume.

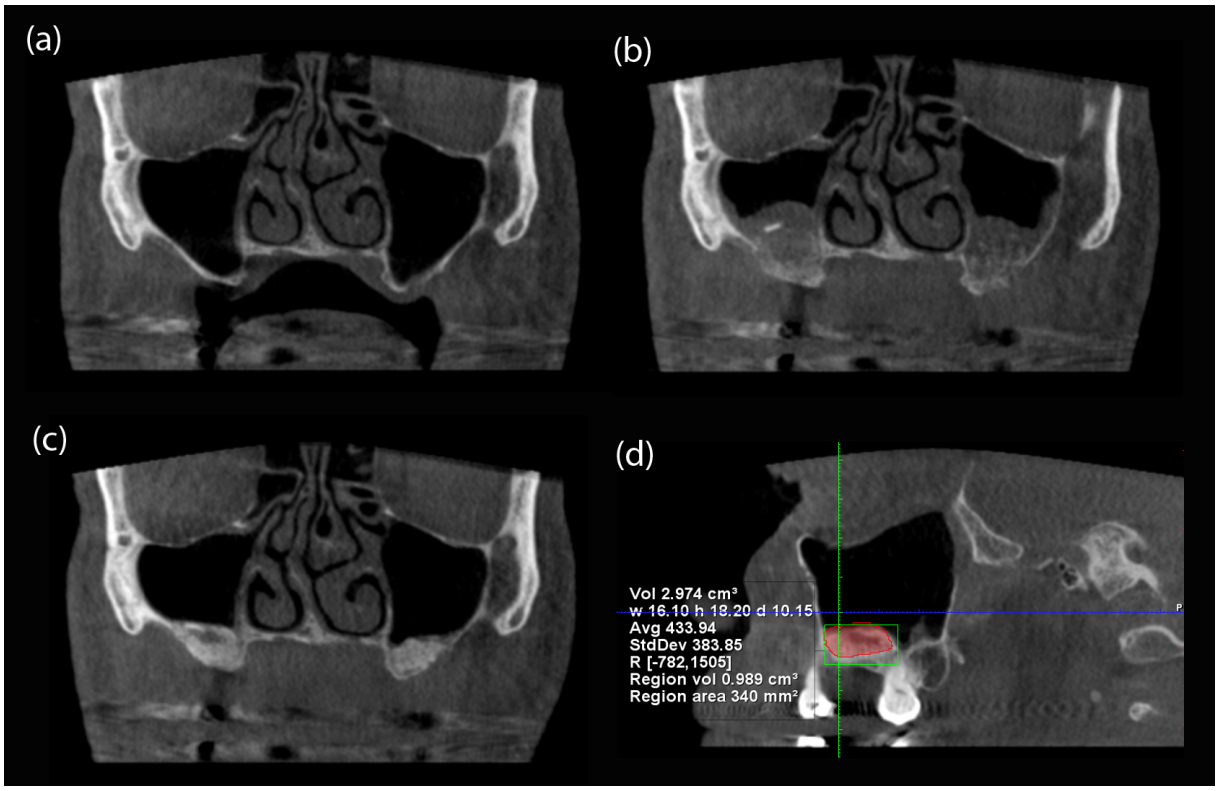


Figure 3- Distribution of volumetric changes (cm^3) immediately after sinus augmentation (T1) and 8 months after the surgery (T2), and percentage of graft volumetric reduction for the control group.

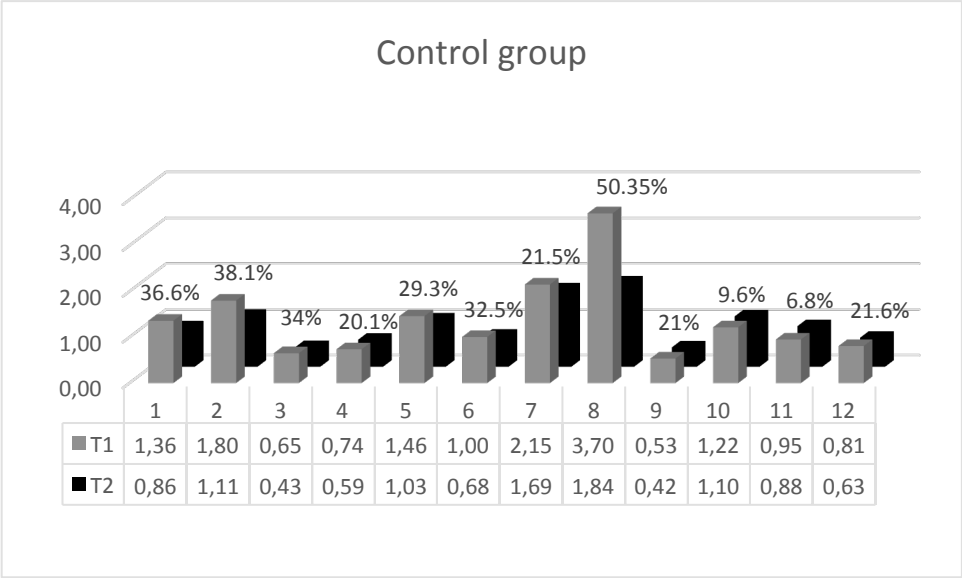


Figure 4- Distribution of volumetric changes (cm^3) immediately after sinus augmentation (T1) and 8 months after the surgery (T2), , and percentage of graft volumetric reduction for the test group.

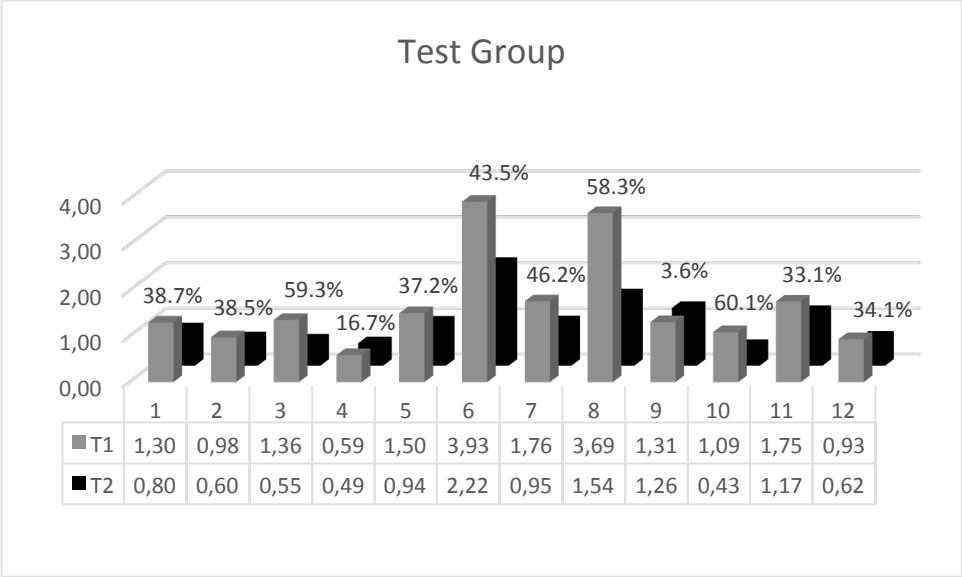


Figure 5- Mean (SD) percent reduction in bone volume graft in control (n=12) and test (n=12) groups after 8 months of healing; with statistically significant difference between groups (*$P < 0.05$).

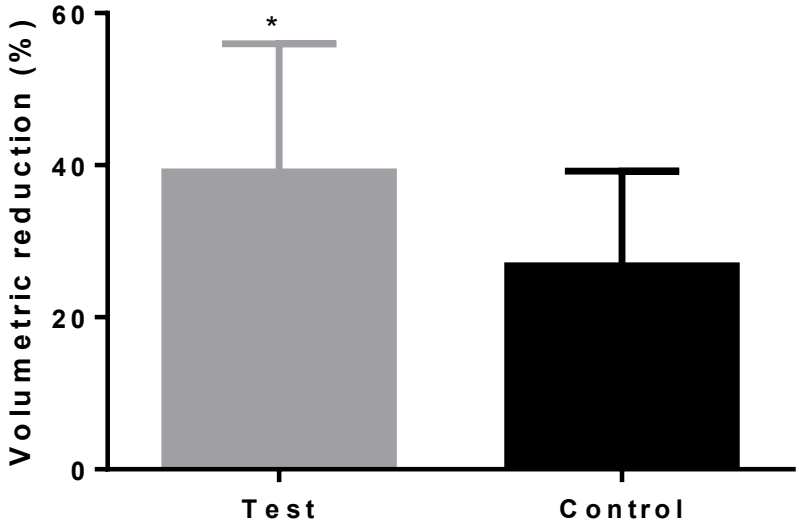
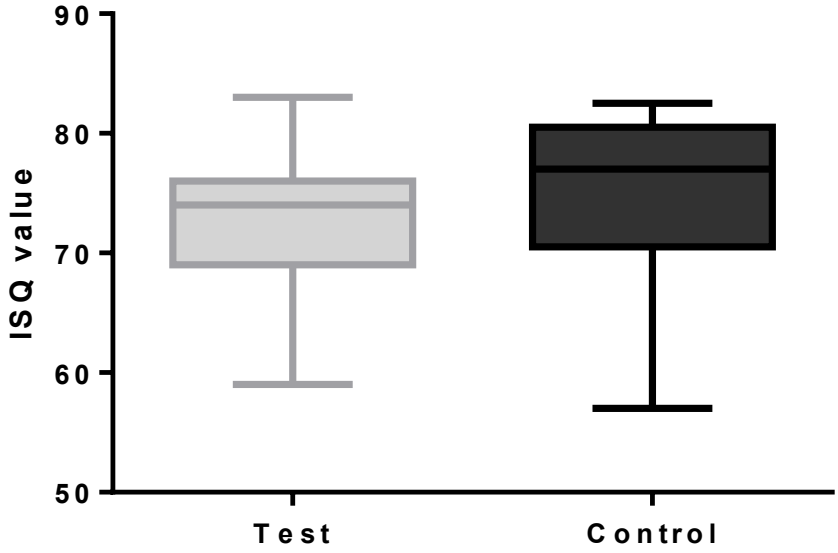


Figure 6 – Boxplot of the implant stability quotient (ISQ) values obtained for control (n=12) and test (n=12) groups after implant installation; no statistically significant difference was found between groups ($P > 0.05$).



3.2 Publicação 2

Maxillary sinus augmentation with leukocyte and platelet-rich fibrin (L-PRF) and deproteinized bovine bone mineral for bone regeneration improvement at the implant site. . A double blinded, randomized-controlled, prospective, and split-mouth clinical trial

Running title: Sinus augmentation with L-PRF and DBBM for bone regeneration improvement

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ABSTRACT

Objectives: Leukocyte and platelet-rich fibrin (L-PRF) is an autologous fibrin matrix rich in growth factors that has been widely used in bone regeneration procedures. However, its benefits are still unclear. The aim of this randomized clinical trial was to evaluate whether or not L-PRF improves bone regeneration in maxillary sinus augmentation when associated with deproteinized bovine bone mineral (DBBM).

Materials and Methods: Twelve totally or partially edentulous patients (nine females and three males, mean age ± SD; 53.92 ± 12.74) requiring bilateral maxillary sinus floor augmentation were enrolled. The inclusion criteria involved maxillary atrophy with ≤ 4 mm of residual ridge. A split-mouth design was employed, in which the maxillary sinuses in the test group were grafted with a mixture of DBBM and L-PRF, and the control group was grafted with DBBM only. The sinuses were randomly allocated to each group and a single operator performed the same surgical procedures in both groups. After 8 months of healing, bone biopsies were harvested from the implant sites for histological and histomorphometric evaluations.

Results: Both techniques were effective for maxillary sinus augmentation. Nineteen implants of 4.0 mm diameter and 11 mm length were installed in each group 8 months post-operatively. Statistically significant difference in the total amount of newly formed bone was observed between the test (2.45 ± 0.64 mm2) and the control group (1.81 ± 0.41 mm^2). A higher percentage of newly formed bone was observed in the test group (46.5 ± 12.27%) compared to the control group (34.51 ± 7.81%) ($P < 0.05$). The percentages of residual graft material and fibrous tissue were similar for both groups.

Conclusions: The application of L-PRF in combination with DBBM in maxillary sinus augmentation significantly improved the amount of regenerated bone after 8 months of healing.

Key words: Bone substitutes, cone beam computed tomography, implants, maxillary sinus; sinus floor augmentation; titanium

1. INTRODUCTION

Sinus floor augmentation plays a pivotal role for the installation of appropriate implant length when the posterior maxilla is severely resorbed (Kahnberg, et al. 2001). Sinus lift described by Boyne and James (Boyne & James 1980) and developed by Tatum (Tatum 1986) has been proposed as a safe and one of the most frequently performed treatment approach for rehabilitation of atrophic maxilla in the posterior region (Dos Anjos, et al. 2016, Hallman, et al. 2005, Summers 1994, Wetzel, et al. 1995, Zitzmann & Scharer 1998).

Autogenous bone remains as the gold standard graft material for sinus augmentation procedure. The literature has reported high implant success rates in maxillary sinus grafted with autogenous bone (Lutz, et al. 2015, Misch & Dietsh 1993, Xavier, et al. 2015). The advantages of autogenous bone are the same embryogenetic derivation, the presence of bone morphogenetic protein, and viable cells capable to induce bone neoformation (osteogenic properties) (Khairy, et al. 2013, Tatullo, et al. 2012). However, the limited source of autogenous bone and the highly patient morbidity are drawbacks related to this approach.

Demineralized bovine bone mineral (DBBM) is a well documented osteoconductive biomaterial with high clinical success rate in sinus augmentation procedures and implant survival (Piattelli, et al. 1999, Rosen, et al. 2002, Traini, et al. 2007, Valentini, et al. 2000, Valentini & Abensur 2003, Wallace, et al. 2005). It is usually used alone or in combination with autologous bone (Hallman, Sennerby, Zetterqvist & Lundgren 2005, Valentini, et al. 1998, Yildirim, et al. 2001) due to the biocompatibility and lower resorbable rate (Artzi, et al. 2001, Simion, et al. 2007).

The granular spaces of the DBBM allow the cellular aggregation with deposition of new bone surrounding the biomaterial. However, the lack of osteoinductive property is a disadvantage of DBBM. To overcome this problem, the addition of growth factors to biomaterials have been proposed in an attempt to improve bone healing and increase bone formation (Castro, et al. 2017, Nizam, et al. 2017, Simonpieri, et al. 2012).

A new conception of platelet concentrate was first described by Choukroun et al. (Choukroun, et al. 2006) and was classified as a leukocyte and platelet rich fibrin (L-PRF) concentrate. It has been describe as an autologous biomaterial that contains many growth factors such as platelet-derived growth factor (PGDF), transforming growth factor (TGF-β), vascular endothelial growth factor (VEGF) and insulin-like growth factor (IGF) (Marx, et al. 1998, Weibric, et al. 2002).

To obtain the L-PRF from the patient, the blood is collected without any anticoagulant, carrier or activator, which makes the technique simple and inexpensive (Dohan Ehrenfest, et al. 2009). L-PRF is successfully used when mixed with graft materials in intrabone periodontal defect (Chang & Zhao 2011, Thorat, et al. 2011), peri-implant defects (Lee, et al. 2012), socket preservation (Simon, et al. 2009) and sinus lifting surgery (Choukroun, Diss, Simonpieri, Girard, Schoeffler, Dohan, Dohan, Mouhyi & Dohan 2006, Kim, et al. 2012, Mazor, et al. 2009, Nizam, Eren, Akcali & Donos 2017, Simonpieri, et al. 2011, Zhang, et al. 2012).

In a clinical study (Tatullo, Marrelli, Cassetta, Pacifici, Stefanelli, Scacco, Dipalma, Pacifici & Inchingolo 2012), PRF enhanced bone regeneration and enabled successfully endosseous implant placement after 106 days using DBBM associated with PRF. The healing time was significant reduced and the histological evaluation revealed the same bone quality when it was compare with 8 months using only DBBM. On the other hand, recent studies (Nizam, Eren, Akcali & Donos 2017, Zhang, Tangl, Huber, Lin, Qiu & Rausch-Fan 2012) did

not show differences in the percentage of newly formed bone, residual bone graft and soft tissue when L-PRF was added to the DBBM in sinus augmentation procedure.

Although acceptable clinical outcomes using different biomaterials have been demonstrated in sinus floor augmentation (Corbella, et al. 2016, Nizam, Eren, Akcali & Donos 2017) there is still a gap in our knowledge about the real capability of the L-PRF in accelerate bone healing and to enhance bone regeneration when associated with DBBM. Therefore, the aim of this clinical trial was to evaluate histologically the effects of leukocyte and platelet-rich fibrin (L-PRF) in combination with deproteinized bovine bone mineral (DBBM) in maxillary sinus augmentation.

2. MATERIAL AND METHODS

This randomized, controlled, prospective, double-blinded, split-mouth clinical trial was conducted in accordance with the Consolidated Standards of Reporting Trials (CONSORT) Statement (Schulz, et al. 2010).The study protocol was approved by the Ethical Committee on Human Research (protocol number #41357514.5.0000.5416) before patient enrolment. All patients were informed about the surgical procedures and a written informed consent were obtained from all subjects.

Patients and study design

Patients were recruited at the Department of Diagnosis and Oral Surgery of the School of Dentistry at Araraquara – UNESP from May 2015 through August 2016. A total of 12 patients were enrolled in this study; nine were female and three were male, and they ranged in age from 26 to 69 years (with mean age of 54 years). Nine patients were partially edentulous and three patients were totally edentulous in the upper jaw. The inclusion criteria were as follows: patients who required bilaterally sinus floor augmentation for implant installation in the posterior maxillary region with residual bone height of < 5 mm (based on CBCT). The

exclusion criteria included: compromised general healthy condition, smokers or ex-smokers, alcohol and drug abusers, irradiated patients, pregnancy, therapies with bisphosphonates, blood platelet disorders, chronic sinusitis, patients suffering from any pathology in the maxillary sinus, and uncontrolled diabetes (de Molon, et al. 2013).

All subjects included in this study were assigned randomly by means of a computer generated randomization list to two experimental groups to be grafted with L-PRF and DBBM (test group) or a control group grafted with DBBM (Bio-Oss®, Geistlich Pharma AG, Switzerland) only.

PRF preparation

The L-PRF clothes were prepared according to the technique described by Choukroun et al. (Choukroun, Diss, Simonpieri, Girard, Schoeffler, Dohan, Dohan, Mouhyi & Dohan 2006). Venous blood samples were taken at the beginning of the procedure using vacutainers (BD, Franklin Lakes, NJ, USA) and centrifuged at 3000 rpm (300 × g) for 10 minutes (Kasvi K14-0815, Curitiba, PR, Brazil). After centrifugation, L-PRF was present in the middle of the tube, between the acellular plasma at the top and the red corpuscle at the bottom. Form each cloth, one L-PRF membrane was obtained.

Sinus augmentation procedure

The surgical procedure started with intra and extra oral antisepsis with 0.12% and 2% of chlorhexidine digluconate, respectively. Under local anesthesia (Articaine 4% and epinephrine 1:100,000; DFL, Rio de Janeiro, RJ, Brazil) a mid-crestal and vertical releasing incisions were performed along the residual alveolar bone to expose the lateral sinus wall. A lateral window approach was performed to access the sinus wall using diamond round bur in a high-speed hand piece (KaVo do Brasil; S.A. Ind. e Com. Joinvile, SC, Brazil) with sterile saline irrigation, as described previously (de Molon, et al. 2015, Dos Anjos, de Molon, Paim,

Marcantonio, Marcantonio & Faeda 2016). The surgical access respected the position of implant placement planning and the maxillary sinus anatomy. After carefully sinus membrane elevation, the control side was grafted with small particles (0.25-1 mm) of DBBM (Bio-Oss®, Geistlich Pharma AG, Wolhusen, Switzerland), while the test side was grafted with a mixture of L-PRF membranes and DBBM (0.25-1 mm). The L-PRF membranes were cuted into small pieces and mixed with DBBM in the following: 0.5 g of Bio-Oss® was added to each L-PRF membrane (4-5 mL). The graft materials were gently compacted at the sinus cavity. A resorbable collagen membrane (Bio-Gide®, Geistlich Pharma AG, Wolhusen, Switzerland) was used to cover the lateral window. The mucosal flap was replaced with 4-0 nylon (Ethicon, Jonhson & Jonhson S.A. Sao Paulo, SP, Brazil).

After surgery, the patients received postoperative instructions for appropriate oral hygiene control, oral antibiotics (amoxicillin, 500 mg three times a day for a week), oral anti-inflammatory (nimesulide, 100 mg twice a day for 5 days), and analgesic (paracetamol, 750 mg every 6 hours for 2 days). They were advised to rinse their mouth with chlorhexidine (0.2%) for 14 days. The sutures were removed 7 days after the surgical procedure, and the area was not subjected to any direct loading during the entire bone regeneration phase.

All patients were followed during the healing phase to evaluate and prevent any complications after surgery such as: inflammation control, wound dehiscence and loss of grafted bone particles.

Implant placement

After 8 months of healing, the same surgeon performed the second surgery bilaterally for dental implants insertion in the posterior maxilla region. A total of 38 implants (TitamaxTi EX ACQUA, Neodent, Curitiba, Brazil) of 4 mm in diameter and 11 mm in length were placed, 19 in the control side and 19 in the test side

Histology and histomorphometric analysis

During implant site preparation, bone biopsies were harvested from the maxillary sinus with the aid of a trephine drill (3i Implant Innovations, Florida, FL, USA) (3.0 mm in diameter and 15 mm in length). Two cylinder bone biopsies were obtained per patient (one for each sinus) and the trephine sites were used for implant placement. The biopsy involved the residual maxillary bone and augmented sinus (bone graft), and preparation depth was defined from the planned implant length. After biopsies removal, the implants were placed.

Biopsies were immediately fixed in 10% buffered formaldehyde solution for 3 days and then processed, as described elsewhere (de Molon, de Paula, Spin-Neto, Verzola, Tosoni, Lia, Scaf & Marcantonio 2015). Serial sections of 6-μm thickness were obtained using an automatic microtome (Jung Supercut 2065, Leica Instruments GmbH, Heidelberg, Germany), mounted on slides and stained with hematoxylin and eosin (H/E). The histological evaluation was made using an optical microscope (Diastar; Leica microsystems GmbH, Wetzlar, Germany) at 100× magnification. Images were selected and transferred to a computer display through a digital camera attached to the optical microscope (DFC-300-FX, Leica microsystems GmbH, Wetzlar, Germany) allowing histomorphometric analysis.

A blinded examiner performed the histomorphometric analysis. The digital images of histological slides were imported and analyzed into the program Image J for Windows (Image J 1.45; Wayne Rasband National Institutes of Health, USA). The histomorphometric analysis was perfomed to measure the newly formed bone, the percentage of new bone formation, the residual bone graft, and the amount of fibrous tissue 8 months after sinus floor augmentation. Selected slides for histomorphometric analysis followed the semi-series standard: the first section of the first slide was selected, and then four sections sequencing were despised and so on. Ten sections were evaluated from each specimen.

Statistical analysis

The minimum sample size calculation for this study was calculated using G*Power 3.1 (Faul, et al. 2009). Considering a standard deviation of 5.0% for the primary outcome (percentage of newly formed bone) and a mean difference of 5.5% between the two experimental groups, an effect size of 1.1 was obtained. The effect size was calculated based on previous data (Zhang, Tangl, Huber, Lin, Qiu & Rausch-Fan 2012). Using this effect size with a given alpha level of 0.05 and a power of 80% and an allocation ratio of 1, a sample size of 9 patients per group was calculated.

Statistical analysis was performed using the GraphPad Prism software (version 6.0, GraphPad Software, Inc., La Jolla, CA, USA). All data were expressed as the mean ± the standard error of the mean (SEM). All data were submitted to the D'Agostino & Pearson test to assess the normality of data distribution. All parameters for histomorphometric analyses did not show normal distribution, and therefore Wilcoxon test was used to access the differences between groups. Differences were considered significant at $P < 0.05$.

3. RESULTS

Patient characteristics

Maxillary sinus floor augmentation was performed bilaterally in twelve patients. One side, randomly selected, was filled with L-PRF + DBBM and the contralateral side was grafted with DBBM only. Implants were installed after 8 months post-operative. During the healing period, the patients did not wear any provisional removable denture. After the healing period, 38 implants were installed, 19 in the control group, and 19 in the test group. No complications were observed during or after the sinus augmentation procedure. No perforation of the sinus membrane was verified, and none of the implants inserted was lost during the follow-up period.

Bone histomorphometry

Before implant installation, bone biopsies were harvested from the maxillary sinus for histomorphometric analysis. The data showed statistically significant increase in the amount of newly formed bone (Fig. 1A) between test (2.45 ± 0.64 mm^2) and control group (1.81 ± 0.41 mm^2). Accordingly, the percentage of new bone formation (Fig. 1B) were significantly increased in the test group (46.5 ± 12.27%) compared to the control group (34.51 ± 7.81%) ($P < 0.05$).

No statistically significant differences were found between the test (0.36 ± 0.44 mm^2) and control (0.52 ± 0.35 mm^2) groups for the amount of residual graft material (Fig. 2A). As expected, the percentage of bone graft (Fig. 2B) between groups were not statistically significant (7.01 ± 8.48% and 9.95 ± 6.65% for the test and control groups, respectively). In regard to the amount of fibrous tissue (Fig. 3A) in the maxillary sinus cavities, the results were also not significant between groups. The control group showed slight increase in the amount of fibrous tissue (1.21 ± 0.60 mm^2) compared to the test group (0.93 ± 0.63 mm^2). Similarly, the percentage of fibrous tissue (Fig. 3B) were also not different between groups (23.09 ± 11.47% and 17.76 ± 12.03% for the control and test groups, respectively).

4. DISCUSSION

This study investigate whether the addition of L-PRF to the DBBM graft would increase bone regeneration in maxillary sinus augmentation procedure. Our findings demonstrate that the addition of L-PRF to the DBBM graft resulted in increased percentage and volume of newly formed bone in maxillary sinus of 12 patients after 8 months of healing. Moreover, both approaches had similar effect in terms of the amount and percentage of residual bone graft and fibrous tissue, although a slightly increase in graft resorption was observed when L-PRF was added to the DBBM. Furthermore, there were no postoperative complications, and the success rate for implant survival was 100%.

Bio-Oss (Geistlich Pharma AB) is a low-resorbable DBBM, presenting 75–80% porosity, in the form of cortical granules and a large-mesh interconnecting system acting as a scaffold that allows angiogenesis, osteoblast cell migration, and consequently the formation of new bone (Artzi, et al. 2004). In an attempt to enhance bone formation and accelerates bone healing in maxillary sinus floor augmentation, the addition of L-PRF to the DBBM has been suggested (Nizam, Eren, Akcali & Donos 2017, Zhang, Tangl, Huber, Lin, Qiu & Rausch-Fan 2012). The rational of adding L-PRF to the DBBM graft is based in previous study that demonstrated improved proliferation of human osteoblasts and increased alkaline phosphatase activity (Dohan Ehrenfest, Rasmusson & Albrektsson 2009). It has also been demonstrated that platelets have favorable effects on hard tissue healing by means of release of growth factors (Chang, et al. 2010), which might help promote angiogenesis and osteogenesis (De Pascale, et al. 2015). Additionally, a clinical study (Choukroun, Diss, Simonpieri, Girard, Schoeffler, Dohan, Dohan, Mouhyi & Dohan 2006) demonstrated accelerate bone regeneration when L-PRF was added to the freeze-dried bone allograft.

A recent study investigated whether the addition of L-PRF to the DBBM graft would enhance bone formation by means of histological analysis (Nizam, Eren, Akcali & Donos 2017). Thirteen patients underwent bilaterally sinus floor augmentation and bone biopsies were collected six months after healing period before implant installation. The results did not show any difference in terms of newly formed bone (21.38 ± 8.78% in the L-PRF group and 21.25 ± 5.59% in the DBBM group), neither the amount of residual graft (L-PRF; 25.95 ± 9.54% and DBBM; 32.79 ± 5.89%) and soft tissue (L-PRF 52.67 ± 12.53% and DBBM 45.96 ± 8.36%). Paralleling clinical data, Zhang et al. (Zhang, Tangl, Huber, Lin, Qiu & Rausch-Fan 2012) demonstrated similar outcomes when adding L-PRF i.e., no differences in the percentage of new bone formation (DBBM + L-PRF 18.35% ± 5.62% vs. DDDM 12.95% ± 5.33%), while the percentage of residual bone graft in the DBBM group was slightly higher as that in the DBBM + L-PRF group (28.54% ± 12.01% vs. 19.16% ± 6.89%). These

contradictory results found in the literature compared to our study could be attributed to the differences in the study design, patient selection, healing period, residual bone height, appropriate application of indicated surgical techniques, and sample size.

The favorable effects of L-PRF on bone regeneration by means of release of growth factors, and enhancement of angiogenesis and osteogenesis might explain the higher percentage of newly formed bone in the maxillary sinus, which corroborates the findings of a recent systematic review (Castro, Meschi, Temmerman, Pinto, Lambrechts, Teughels & Quirynen 2017).

Although no statistically difference was found in regard to the amount of residual bone graft between both groups (7.01 ± 8.48% for test group and 9.95 ± 6.65% for the control group), the slightly increased in residual bone graft in the sinus filled with only DBBM might be explained by the lower amount of DBBM applied to the sinus when associated with the L-PRF, and also to the L-PRF degradation over time. Paralleling clinical data from previous study (Kirmeier, et al. 2008), the resorption rate when DBBM is used vary between 13,9 and 26% after 6 months post-surgery, which close resemble our findings. According to a previous study (Kim, et al. 2013), there is no significant relationship between the resorption of grafted bone and the implant success rate (osseointegration) due to the increased stability of the graft over time, especially after 12 months post-operative.

5. CONCLUSIONS

Collectively, our finding demonstrated that the combination of L-PRF and DBBM resulted in significant increase of newly formed bone after maxillary sinus augmentation procedure. No differences were found in regard to the amount and percentage of residual graft material and soft tissue. Taken together, both groups performed well and allowed adequate implant length installation after maxillary sinus augmentation.

REFERENCES

Artzi, Z., Tal, H. & Dayan, D. (2001) Porous bovine bone mineral in healing of human extraction sockets: 2. Histochemical observations at 9 months. *J Periodontol* **72**: 152-159.

Artzi, Z., Weinreb, M., Givol, N., Rohrer, M. D., Nemcovsky, C. E., Prasad, H. S. & Tal, H. (2004) Biomaterial resorption rate and healing site morphology of inorganic bovine bone and beta-tricalcium phosphate in the canine: A 24-month longitudinal histologic study and morphometric analysis. *Int J Oral Maxillofac Implants* **19**: 357-368.

Boyne, P. J. & James, R. A. (1980) Grafting of the maxillary sinus floor with autogenous marrow and bone. *J Oral Surg* **38**: 613-616.

Castro, A. B., Meschi, N., Temmerman, A., Pinto, N., Lambrechts, P., Teughels, W. & Quirynen, M. (2017) Regenerative potential of leucocyte- and platelet-rich fibrin. Part b: Sinus floor elevation, alveolar ridge preservation and implant therapy. A systematic review. *J Clin Periodontol* **44**: 225-234.

Chang, I. C., Tsai, C. H. & Chang, Y. C. (2010) Platelet-rich fibrin modulates the expression of extracellular signal-regulated protein kinase and osteoprotegerin in human osteoblasts. *J Biomed Mater Res A* **95**: 327-332.

Chang, Y. C. & Zhao, J. H. (2011) Effects of platelet-rich fibrin on human periodontal ligament fibroblasts and application for periodontal infrabony defects. *Aust Dent J* **56**: 365-371.

Choukroun, J., Diss, A., Simonpieri, A., Girard, M. O., Schoeffler, C., Dohan, S. L., Dohan, A. J., Mouhyi, J. & Dohan, D. M. (2006) Platelet-rich fibrin (prf): A second-generation platelet concentrate. Part v: Histologic evaluations of prf effects on bone allograft maturation in sinus lift. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **101**: 299-303.

Corbella, S., Taschieri, S., Weinstein, R. & Del Fabbro, M. (2016) Histomorphometric outcomes after lateral sinus floor elevation procedure: A systematic review of the literature and meta-analysis. *Clin Oral Implants Res* **27**: 1106-1122.

de Molon, R. S., de Paula, W. N., Spin-Neto, R., Verzola, M. H., Tosoni, G. M., Lia, R. C., Scaf, G. & Marcantonio, E., Jr. (2015) Correlation of fractal dimension with histomorphometry in maxillary sinus lifting using autogenous bone graft. *Braz Dent J* **26**: 11-18.

de Molon, R. S., Morais-Camilo, J. A., Verzola, M. H., Faeda, R. S., Pepato, M. T. & Marcantonio, E., Jr. (2013) Impact of diabetes mellitus and metabolic control on bone healing around osseointegrated implants: Removal torque and histomorphometric analysis in rats. *Clin Oral Implants Res* **24**: 831-837.

De Pascale, M. R., Sommese, L., Casamassimi, A. & Napoli, C. (2015) Platelet derivatives in regenerative medicine: An update. *Transfus Med Rev* **29**: 52-61.

Dohan Ehrenfest, D. M., Rasmusson, L. & Albrektsson, T. (2009) Classification of platelet concentrates: From pure platelet-rich plasma (p-prp) to leucocyte- and platelet-rich fibrin (l-prf). *Trends Biotechnol* **27**: 158-167.

Dos Anjos, T. L., de Molon, R. S., Paim, P. R., Marcantonio, E., Marcantonio, E., Jr. & Faeda, R. S. (2016) Implant stability after sinus floor augmentation with deproteinized bovine bone mineral particles of different sizes: A prospective, randomized and controlled split-mouth clinical trial. *Int J Oral Maxillofac Surg* **45**: 1556-1563.

Faul, F., Erdfelder, E., Buchner, A. & Lang, A. G. (2009) Statistical power analyses using g*power 3.1: Tests for correlation and regression analyses. *Behav Res Methods* **41**: 1149-1160.

Hallman, M., Sennerby, L., Zetterqvist, L. & Lundgren, S. (2005) A 3-year prospective follow-up study of implant-supported fixed prostheses in patients subjected to maxillary sinus floor augmentation with a 80:20 mixture of deproteinized bovine bone and autogenous bone clinical, radiographic and resonance frequency analysis. *Int J Oral Maxillofac Surg* **34**: 273-280.

Kahnberg, K. E., Ekestubbe, A., Grondahl, K., Nilsson, P. & Hirsch, J. M. (2001) Sinus lifting procedure. I. One-stage surgery with bone transplant and implants. *Clin Oral Implants Res* **12**: 479-487.

Khairy, N. M., Shendy, E. E., Askar, N. A. & El-Rouby, D. H. (2013) Effect of platelet rich plasma on bone regeneration in maxillary sinus augmentation (randomized clinical trial). *Int J Oral Maxillofac Surg* **42**: 249-255.

Kim, B. J., Kwon, T. K., Baek, H. S., Hwang, D. S., Kim, C. H., Chung, I. K., Jeong, J. S. & Shin, S. H. (2012) A comparative study of the effectiveness of sinus bone grafting with recombinant human bone morphogenetic protein 2-coated tricalcium phosphate and platelet-rich fibrin-mixed tricalcium phosphate in rabbits. *Oral Surg Oral Med Oral Pathol Oral Radiol* **113**: 583-592.

Kim, E. S., Moon, S. Y., Kim, S. G., Park, H. C. & Oh, J. S. (2013) Three-dimensional volumetric analysis after sinus grafts. *Implant Dent* **22**: 170-174.

Kirmeier, R., Payer, M., Wehrschuetz, M., Jakse, N., Platzer, S. & Lorenzoni, M. (2008) Evaluation of three-dimensional changes after sinus floor augmentation with different grafting materials. *Clin Oral Implants Res* **19**: 366-372.

Lee, J. W., Kim, S. G., Kim, J. Y., Lee, Y. C., Choi, J. Y., Dragos, R. & Rotaru, H. (2012) Restoration of a peri-implant defect by platelet-rich fibrin. *Oral Surg Oral Med Oral Pathol Oral Radiol* **113**: 459-463.

Lutz, R., Berger-Fink, S., Stockmann, P., Neukam, F. W. & Schlegel, K. A. (2015) Sinus floor augmentation with autogenous bone vs. A bovine-derived xenograft - a 5-year retrospective study. *Clin Oral Implants Res* **26**: 644-648.

Marx, R. E., Carlson, E. R., Eichstaedt, R. M., Schimmele, S. R., Strauss, J. E. & Georgeff, K. R. (1998) Platelet-rich plasma: Growth factor enhancement for bone grafts. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **85**: 638-646.

Mazor, Z., Horowitz, R. A., Del Corso, M., Prasad, H. S., Rohrer, M. D. & Dohan Ehrenfest, D. M. (2009) Sinus floor augmentation with simultaneous implant placement using choukroun's platelet-rich fibrin as the sole grafting material: A radiologic and histologic study at 6 months. *J Periodontol* **80**: 2056-2064.

Misch, C. E. & Dietsh, F. (1993) Bone-grafting materials in implant dentistry. *Implant Dent* **2**: 158-167.

Nizam, N., Eren, G., Akcali, A. & Donos, N. (2017) Maxillary sinus augmentation with leukocyte and platelet-rich fibrin and deproteinized bovine bone mineral: A split-mouth histological and histomorphometric study. *Clin Oral Implants Res*.

Piattelli, M., Favero, G. A., Scarano, A., Orsini, G. & Piattelli, A. (1999) Bone reactions to anorganic bovine bone (bio-oss) used in sinus augmentation procedures: A histologic long-term report of 20 cases in humans. *Int J Oral Maxillofac Implants* **14**: 835-840.

Rosen, V. B., Hobbs, L. W. & Spector, M. (2002) The ultrastructure of anorganic bovine bone and selected synthetic hyroxyapatites used as bone graft substitute materials. *Biomaterials* **23**: 921-928.

Schulz, K. F., Altman, D. G., Moher, D. & Group, C. (2010) Consort 2010 statement: Updated guidelines for reporting parallel group randomised trials. *BMC Med* **8**: 18.

Simion, M., Fontana, F., Rasperini, G. & Maiorana, C. (2007) Vertical ridge augmentation by expanded-polytetrafluoroethylene membrane and a combination of intraoral autogenous bone graft and deproteinized anorganic bovine bone (bio oss). *Clin Oral Implants Res* **18**: 620-629.

Simon, B. I., Zatcoff, A. L., Kong, J. J. & O'Connell, S. M. (2009) Comparison of extraction socket healing following the use of autologous platelet- rich fibrin matrix (prfm) to ridge preservation procedures employing demineralized freeze dried bone allograft material and membrane. *Open Dent J* **3**: 92-99.

Simonpieri, A., Choukroun, J., Del Corso, M., Sammartino, G. & Dohan Ehrenfest, D. M. (2011) Simultaneous sinus-lift and implantation using microthreaded implants and leukocyte- and platelet-rich fibrin as sole grafting material: A six-year experience. *Implant Dent* **20**: 2-12.

Simonpieri, A., Del Corso, M., Vervelle, A., Jimbo, R., Inchingolo, F., Sammartino, G. & Dohan Ehrenfest, D. M. (2012) Current knowledge and perspectives for the use of platelet-rich plasma (prp) and platelet-rich fibrin (prf) in oral and maxillofacial surgery part 2: Bone graft, implant and reconstructive surgery. *Curr Pharm Biotechnol* **13**: 1231-1256.

Summers, R. B. (1994) A new concept in maxillary implant surgery: The osteotome technique. *Compendium* **15**: 152, 154-156, 158 passim; quiz 162.

Tatullo, M., Marrelli, M., Cassetta, M., Pacifici, A., Stefanelli, L. V., Scacco, S., Dipalma, G., Pacifici, L. & Inchingolo, F. (2012) Platelet rich fibrin (p.R.F.) in reconstructive surgery of atrophied maxillary bones: Clinical and histological evaluations. *Int J Med Sci* **9**: 872-880.

Tatum, H., Jr. (1986) Maxillary and sinus implant reconstructions. *Dent Clin North Am* **30**: 207-229.

Thorat, M., Pradeep, A. R. & Pallavi, B. (2011) Clinical effect of autologous platelet-rich fibrin in the treatment of intra-bony defects: A controlled clinical trial. *J Clin Periodontol* **38**: 925-932.

Traini, T., Valentini, P., Iezzi, G. & Piattelli, A. (2007) A histologic and histomorphometric evaluation of anorganic bovine bone retrieved 9 years after a sinus augmentation procedure. *J Periodontol* **78**: 955-961.

Valentini, P., Abensur, D., Densari, D., Graziani, J. N. & Hammerle, C. (1998) Histological evaluation of bio-oss in a 2-stage sinus floor elevation and implantation procedure. A human case report. *Clin Oral Implants Res* **9**: 59-64.

Valentini, P., Abensur, D., Wenz, B., Peetz, M. & Schenk, R. (2000) Sinus grafting with porous bone mineral (bio-oss) for implant placement: A 5-year study on 15 patients. *Int J Periodontics Restorative Dent* **20**: 245-253.

Valentini, P. & Abensur, D. J. (2003) Maxillary sinus grafting with anorganic bovine bone: A clinical report of long-term results. *Int J Oral Maxillofac Implants* **18**: 556-560.

Wallace, S. S., Froum, S. J., Cho, S. C., Elian, N., Monteiro, D., Kim, B. S. & Tarnow, D. P. (2005) Sinus augmentation utilizing anorganic bovine bone (bio-oss) with absorbable and nonabsorbable membranes placed over the lateral window: Histomorphometric and clinical analyses. *Int J Periodontics Restorative Dent* **25**: 551-559.

Weibric, G., Buch, R. S., Kleis, W. K., Hafner, G., Hitzler, W. E. & Wagner, W. (2002) Quantification of thrombocyte growth factors in platelet concentrates produced by discontinuous cell separation. *Growth Factors* **20**: 93-97.

Wetzel, A. C., Stich, H. & Caffesse, R. G. (1995) Bone apposition onto oral implants in the sinus area filled with different grafting materials. A histological study in beagle dogs. *Clin Oral Implants Res* **6**: 155-163.

Xavier, S. P., Silva, E. R., Kahn, A., Chaushu, L. & Chaushu, G. (2015) Maxillary sinus grafting with autograft versus fresh-frozen allograft: A split-mouth evaluation of bone volume dynamics. *Int J Oral Maxillofac Implants* **30**: 1137-1142.

Yildirim, M., Spiekermann, H., Handt, S. & Edelhoff, D. (2001) Maxillary sinus augmentation with the xenograft bio-oss and autogenous intraoral bone for qualitative improvement of the implant site: A histologic and histomorphometric clinical study in humans. *Int J Oral Maxillofac Implants* **16**: 23-33.

Zhang, Y., Tangl, S., Huber, C. D., Lin, Y., Qiu, L. & Rausch-Fan, X. (2012) Effects of choukroun's platelet-rich fibrin on bone regeneration in combination with deproteinized bovine bone mineral in maxillary sinus augmentation: A histological and histomorphometric study. *J Craniomaxillofac Surg* **40**: 321-328.

Zitzmann, N. U. & Scharer, P. (1998) Sinus elevation procedures in the resorbed posterior maxilla. Comparison of the crestal and lateral approaches. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **85**: 8-17.

FIGURES

Figure 1. CONSORT flowchart of the study

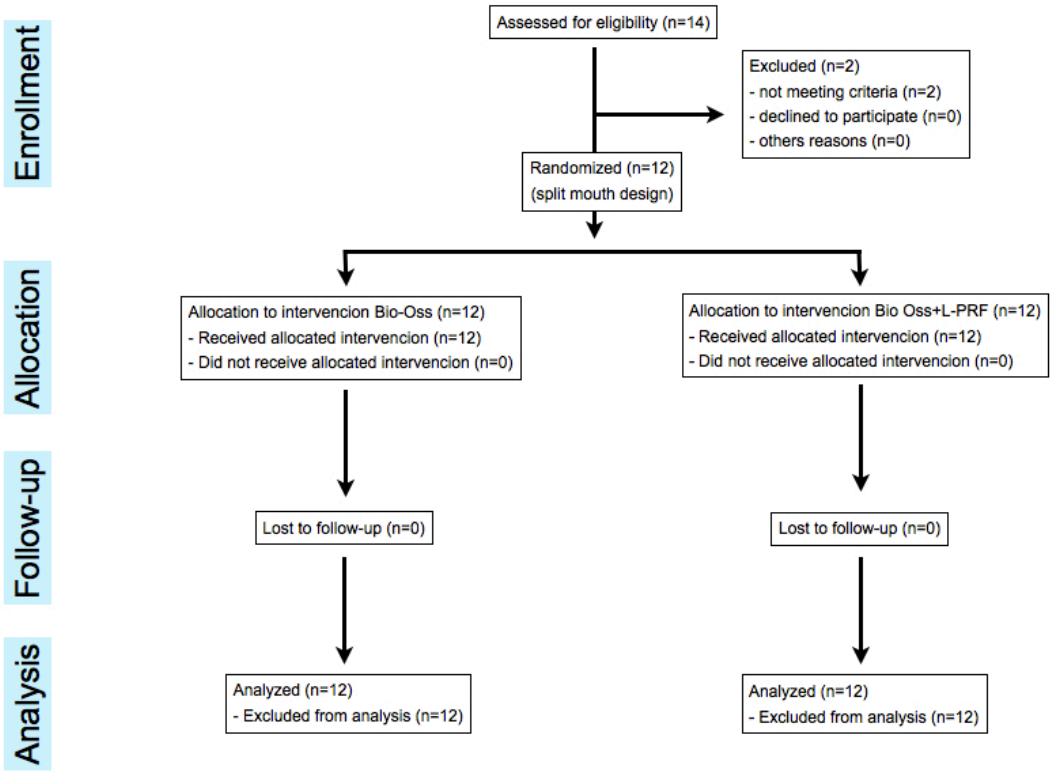


Figure 2. A) Quantification of newly formed bone and **B)** percentage of new bone after maxillary sinus augmentation with the association of L-PRF and DBBM (test group) or only DBBM (control group). *Statistically significant different from indicated group ($P < 0.05$). Differences between groups were calculated by Wilcoxon Test. Data represent the mean ± SEM.

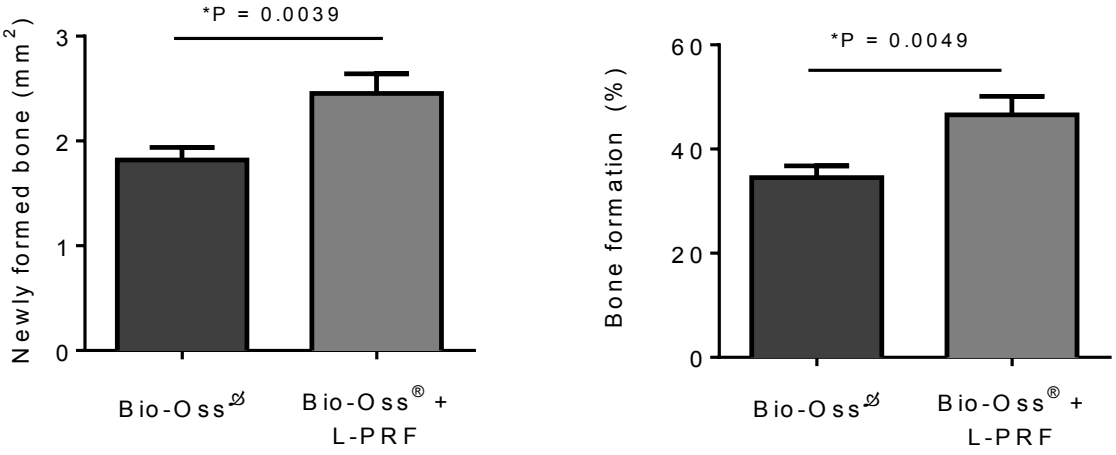


Figure 3. A) Quantification of reminiscent graft material and **B)** percentage of residual graft after maxillary sinus augmentation with the association of L-PRF and DBBM (test group) or only DBBM (control group). Differences between groups were calculated by unpaired t-test. Data represent the mean ± SEM.

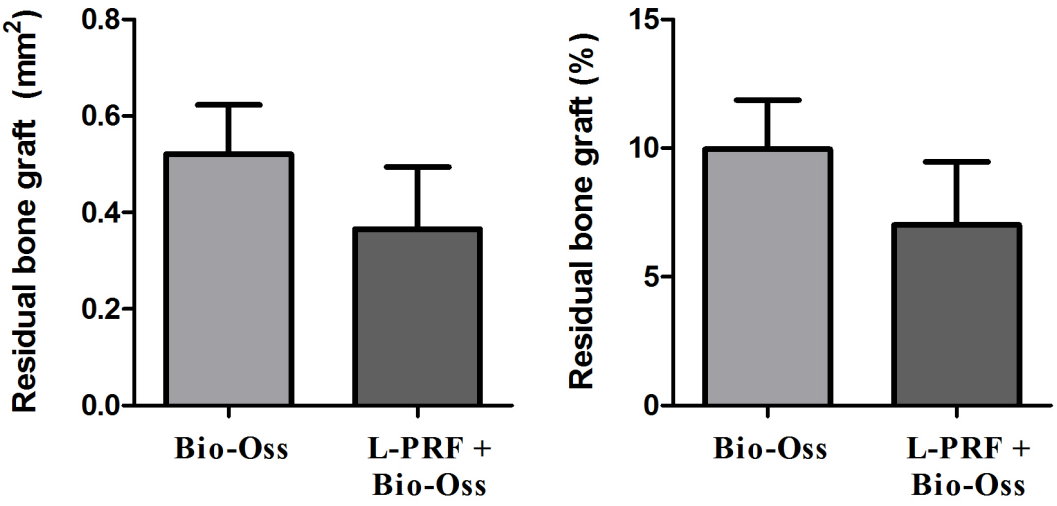
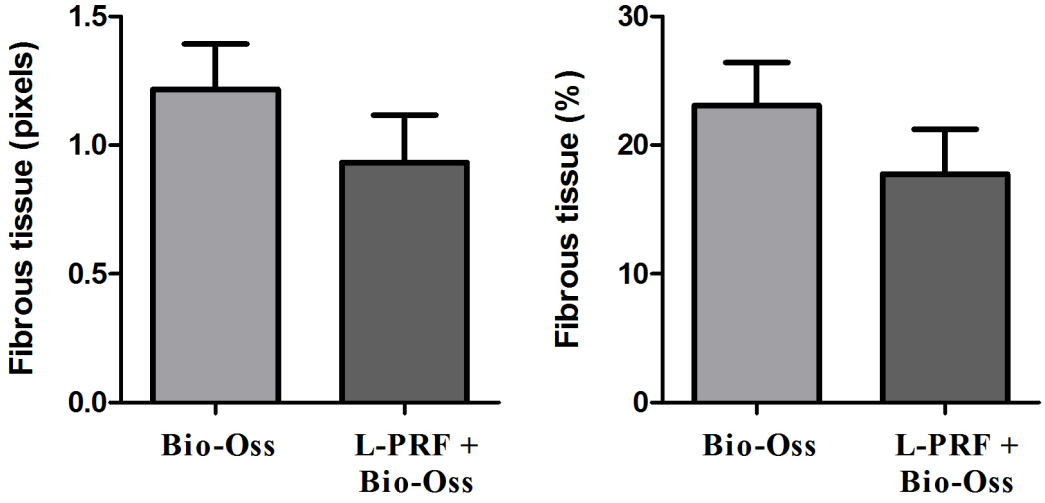


Figure 4. A) Quantification of fibrous tissue and **B)** percentage of fibrous tissue after maxillary sinus augmentation with the association of L-PRF and DBBM (test group) and only DBBM (control group). Differences between groups were calculated by unpaired t-test. Data represent the mean ± SEM.



3.3 Publicação 3

Assessment of the efficiency of leukocyte and platelet-rich fibrin (L-PRF) combined with deproteinized bovine bone graft for early implant placement after maxillary sinus augmentation. A randomized clinical trial[‡]

Running title: Efficiency of L-PRF and DBBM for early implant placement

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ABSTRACT

Objectives: We sought to investigate the efficiency of adding leukocyte and platelet-rich fibrin (L-PRF) with deproteinized bovine bone mineral (DBBM) for early implant placement after maxillary sinus augmentation.

Material and Methods: A prospective, double-blinded, randomized-controlled clinical trial was conducted across twelve subjects requiring 2-stage bilateral maxillary sinus augmentation for dental implant treatment. All patients enrolled presented with residual bone ridge height of ≤ 4 mm, as inclusion criteria. Patients were randomly divided in two groups following a split-mouth design, as follows: test group (DBBM + L-PRF) and control group (only DDBM). After four and eight-months post-operatively, dental implants were inserted in each of the maxillary sinus for the test and control group, respectively. Cone-beam computed tomography (CBCT) was taken before and after sinus augmentation, and after 4 and 8 months of healing for evaluation of tridimensional bone volume alterations. Bone biopsies were gathered during implant placement for histomorphometric evaluation. Resonance frequency analysis (RFA) measured as the clinical expression of implants stability was employed immediately after implant placement for both groups.

Results: CBCT analysis did not reveal differences in bone volume between test and control group for any of the periods evaluated ($P > 0.05$). No dimensional bone changes were evidenced between both groups. Histological evaluation demonstrated increased percentage of newly formed bone for the test group (44.58 ± 13.89%) compared to the control group (30.01 ± 8.41%) (P=0.0087). The amount of residual graft in the control group was significantly higher (13.75 ± 9.99%) than the test group (3.59 ± 4.22) (P=0.0111). Implant stability

quotient (ISQ) was significantly higher in the control group (75.13 ± 5.68) compared to the test group (60.9 ± 9.35) (P=0.0003).

Conclusions: Collectively, our findings demonstrated that the addition of L-PRF to the maxillary sinus allowed early implant placement (4 months) with increased new bone formation compared to the control group 8 months after sinus augmentation. Furthermore, no volumetric bone changes were observed between both groups and increased residual bone graft was evidenced in the control group. However, lower values of ISQ were found for the test group, which did impair implant osseointegration.

Key words: Alveolar bone; bone substitutes, cone-beam computed tomography, dental implants, maxillary sinus; platelet-rich plasma; sinus floor augmentation.

INTRODUCTION

The posterior region of the maxilla possess several challenges for successful dental implant rehabilitation due to the reduced bone quality and by the ridge resorption caused by the sinus pneumatization after teeth loss (Ali et al. 2015). Different approaches for treatment of severely resorbed posterior maxilla have been performed using onlay bone grafts (Cordaro et al. 2010), interpositional grafts after maxillary osteotomy (Pelo et al. 2009), and sinus augmentation procedures (Chiapasco et al. 2009). Sinus lifting with the lateral technique, initially described by Boyne and James (Boyne & James 1980) and established by Tatum (Tatum 1986), is the most commonly used approach to augmentation the maxillary sinus for proper implant length installation. The implant survival rate after sinus lifting is higher than 97% according to Tetsch et al. (Tetsch et al. 2010) and the complication rate are minimal. Furthermore, different graft materials, such as autogenous, (de Molon et al. 2015) xenogenous, (Dos Anjos et al. 2016) alloplastic and the association of bone grafts and growth factors can be safely used during maxillary sinus augmentation (Browaeys et al. 2007,

Scarano et al. 2011). Indeed, this technique has become a routinely treatment modality over the years with highly predictability and effectiveness.

Demineralized bovine bone mineral (DBBM) is frequently used for maxillary sinus augmentation because it possess several advantages compared to the autogenous bone, such as unlimited quantity available, biocompatibility, osteoconductive proprieties (Kazemi et al. 2017), low resorption rate, minimal risk of immunological rejection, lower morbidity to the patient, with a high clinical success rate. Moreover, DBBM act as a scaffold allowing osteogenic cell migration from the maxillary sinus to the graft particles permitting the apposition of de novo bone formation (Bolukbasi et al. 2015, Browaeys; Bouvry & De Bruyn 2007, Tadjoedin et al. 2003). However, DBBM lacks the osteogenic properties, and the maturation of this type of material may take up to 8 months (Choukroun et al. 2006) before implants can be safely installed in maxillary sinus, which might be considered a disadvantage of this material. To overcome this concern, several studies have investigated the addition of growth factor to grafting material aiming at enhance bone neoformation and accelerates graft maturation (Ali; Bakry & Abd-Elhakam 2015, Angelo et al. 2015, Bolukbasi; Ersanli; Keklikoglu; Basegmez & Ozdemir 2015, Boora et al. 2015, Castro et al. 2017, Chang et al. 2010, Choukroun; Diss; Simonpieri; Girard; Schoeffler; Dohan; Dohan; Mouhyi & Dohan 2006, Davis et al. 2014, Davis et al. 2014, Del Corso et al. 2012, Jung et al. 2005, Nizam et al. 2017, Ocak et al. 2017, Oncu & Erbeyoglu 2017, Tabrizi et al. 2017, Tajima et al. 2013).

Leukocyte and platelet rich fibrin (L-PRF), first described by Dohan et al (Dohan et al. 2006), is an autogenous biomaterial containing several growth factors (Ali; Bakry & Abd-Elhakam 2015, Bolukbasi; Ersanli; Keklikoglu; Basegmez & Ozdemir 2015, Choukroun; Diss; Simonpieri; Girard; Schoeffler; Dohan; Dohan; Mouhyi & Dohan 2006, Davis; Abukabda; Radio; Witt-Enderby; Clafshenkel; Cairone & Rutkowski 2014). L-PRF, a second-generation of platelet concentrates, is basically made of concentrated autologous platelets comprising of leukocytes and cytokines (Oncu & Kaymaz 2017). It has been demonstrated

that L-PRF activates the vascular system favoring angiogenesis, and also by releasing several growth factors involved in soft and hard tissue healing (Oncu et al. 2016, Oncu & Erbeyoglu 2017). Additionally, previous study demonstrated that L-PRF is capable of inducing bone regeneration (Castro; Meschi; Temmerman; Pinto; Lambrechts; Teughels & Quirynen 2017, Marx et al. 1998) and fibroblast proliferation (Kumar & Shubhashini 2013) improving and accelerating tissue healing (Oncu; Bayram; Kantarci; Gulsever & Alaaddinoglu 2016), and enhancing implant stability (Oncu & Erbeyoglu 2017). To obtain the L-PRF, the patient's blood is collected through venipuncture without anticoagulant or additives, and it is immediately centrifuged. After processing, L-PRF cloth is localized in the middle of the tube that can either be cut in small pieces and mixed with the DBBM or autogenous bone, or be compressed in two sterile gauze to obtain a fibrin membrane (Bolukbasi; Ersanli; Keklikoglu; Basegmez & Ozdemir 2015). It is a safe, cost-effective and reliable technique to improve repair following surgery (Davis; Abukabda; Radio; Witt-Enderby; Clafshenkel; Cairone & Rutkowski 2014). Due to its dense fibrin fiber network it can perform as scaffold for other cell types because of its stronger mechanical characteristics (Tetsch; Tetsch & Lysek 2010), and also providing support for mesenchymal stem cells (Kang et al. 2011).

Since growth factors play an important role in tissue regeneration, the present study aimed to investigate the effects of L-PRF to accelerate bone healing after maxillary sinus floor augmentation combined with DBBM as graft material, and the outcomes of early placement of dental implants into the maxillary sinus. The study was set up to test the hypothesis that the addition of L-PRF to the graft would enhance new bone formation and early graft maturation speeding implant placement. Bone volume dimensional changes, bone and soft tissue characteristics and implant stability were investigated using CBCT, histology and RFA analysis, respectively.

MATERIAL AND METHODS

This prospective, double-blinded, randomized-controlled clinical trial was accompanied in accordance with the Consolidated Standards of Reporting Trials (CONSORT) Statement (Schulz et al. 2010). The study protocol was approved by the Ethical Committee on Human Research (protocol number #41357514.5.0000.5416) before patient enrolment. All patients were informed about the surgical procedures and written informed consent was obtained from all subjects.

Sample size calculation

To calculate statistical power in this study G*Power 3.1 (Faul et al. 2009) was used. Considering the mean and standard deviation differences between test and control group, an effect size of 2.77 was obtained. Using this effect size, the significance level of 0.05 and the sample size of 12 participants per group, a statistical power of 100% was obtained.

Patients and study design

The study design was based in our previous study. Briefly, patients were recruited at the Department of Diagnosis and Surgery of the School of Dentistry at Araraquara – UNESP from December 2014 through May 2015. A total of 12 patients were enrolled in this study; six patients were male and six were female, and they ranged in age from 43 to 63 years (with mean age of 54.17 ± 6.95 years). Eight patients were partially edentulous and four patients were totally edentulous in the upper jaw. The inclusion criteria were as follows: patients who required bilaterally sinus floor augmentation for implant installation in the posterior maxillary region with residual bone height of < 5 mm (based on CBCT). The exclusion criteria were: compromised general healthy condition, smokers or ex-smokers, alcohol and drug abusers, irradiated patients, pregnancy, therapies with bisphosphonates, blood platelet disorders,

chronic sinusitis, patients suffering from any pathology in the maxillary sinus, and uncontrolled diabetes (de Molon et al. 2013).

All subjects included in this study were assigned randomly by means of a computer generated randomization list to two experimental groups to be grafted with L-PRF and DBBM (test group) or a control group grafted with DBBM (Bio-Oss®, Geistlich Pharma AG, Switzerland) only.

PRF preparation

The L-PRF membranes were prepared according to the technique described by Choukroun et al.(Choukroun; Diss; Simonpieri; Girard; Schoeffler; Dohan; Dohan; Mouhyi & Dohan 2006). Venous blood samples were taken at the beginning of the procedure using vacutainers (BD, Franklin Lakes, NJ, USA) and centrifuged for 10 minutes at 300 x g (Kasvi K14-0815, Curitiba, PR, Brazil). After centrifugation, PRF was present in the middle of the tube, between the acellular plasma at the top and the red corpuscle at the bottom. The L-PRF cloth was squeezed into a metal box (Xpression, Intra-lock Sistem, Sao Paulo, Brazil) to obtain the membrane that was cuted into small pieces and latter mixed with DBBM.

Sinus augmentation procedure

The surgical procedures were performed as described earlier (de Molon; de Paula; Spin-Neto; Verzola; Tosoni; Lia; Scaf & Marcantonio 2015, Dos Anjos; de Molon; Paim; Marcantonio; Marcantonio & Faeda 2016). Briefly, patients received local anesthesia (Articaine 4% and epinephrine 1:100,000; DFL, Rio de Janeiro, RJ, Brazil) followed by a mid-crestal and vertical releasing incisions along the residual alveolar bone to expose the lateral sinus wall. A lateral window approach was performed to access the sinus wall using diamond round bur. The surgical access respected the position of implant placement planning and the maxillary sinus anatomy.

After carefully sinus membrane elevation, the control side was filled with small particles (0.25-1 mm) of DBBM (Bio-Oss®, Geistlich Pharma AG, Wolhusen, Switzerland), while the test side was filled with a mixture of L-PRF membranes and DBBM (0.25-1 mm). For each membrane of L-PRF (4-5 mL) cut in few fragments, 0.5 g of Bio-Oss® was mixed.. The graft materials were gently compacted at the sinus cavity. A resorbable collagen membrane (Bio-Gide®, Geistlich Pharma AG, Wolhusen, Switzerland) was used to cover the lateral window after graft placement, and then soft tissue was sutured.

After surgery, the patients received postoperative instructions for appropriate oral hygiene control, oral antibiotics (amoxicillin, 500 mg three times a day for a week), oral anti-inflammatory (nimesulide, 100 mg twice a day for 5 days), and analgesic (paracetamol, 750 mg every 6 hours for 2 days). They were advised to rinse their mouth with chlorhexidine (0.2%) for 14 days. The sutures were removed 7 days after the surgical procedure, and the area was not subjected to any direct loading during the entire bone regeneration phase.

Implant placement

After 4 months (test group) and 8 months (control) of healing, dental implants were installed in both augmented maxillary sinuses. A total of 38 implants (TitamaxTi EX ACQUA, Neodent, Curitiba, Brazil) were placed, 19 in the control side and 19 in the test side, according to the manufacturer`s protocol. All implants had 4 mm in diameter and 11 mm in length.

Radiographic analysis

Each patient underwent four CBCT scans (SCANORA® 3Dx, Soredex, Tuusula, Finland): preoperative (T0), immediately after maxillary sinus augmentation (T1), after 4 months (T2) and 8 months (T3) post-maxillary sinus augmentation (Harris et al. 2012). The following

parameters were used for all scans, 10 mAs, 90 kVp and a 20 s scan time using the 9 inch field of view (FOV), with a 350 μm voxel size. Preoperative scan was used to evaluate the sinus anatomy and the reminiscent alveolar ridge for implant placement in a two-stage surgery. The immediately post-augmentation scan was performed seven days after the surgery. The 4-month post augmentation scan was performed in the test group before implant placement. The 8-month scan was performed in the control group prior to the implant surgery. The raw data of the scans were reconstructed and exported in DICOM file format and imported in Planmeca software (Planmeca Oy, Helsinki, Finland).

The volumetric measurements of the grafts were determined using sagittal sections of 1 mm through the differential radiodensity of the images. The volumetric dimensions were automatically calculated by the Planmeca software and represented in cubic centimeters. One maxillofacial radiologist experienced with CBCT that received prior training in Planmeca software performed all volumetric measurements in a standardized manner.

Histology and histomorphometric analysis

During implant site preparation, bone biopsies were harvested from the maxillary sinus with the aid of a trephine drill (3i Implant Innovations, Florida, FL, USA.) (3.0 mm in diameter and 15 mm in length). Two-bone biopsies cylinder were obtained per patient (one for each sinus) and the trephine sites were used for implant placement. The biopsy involved the residual maxillary bone and augmented sinus (bone graft), and preparation depth was defined from the planned implant length. After biopsies removal, a total of 38 implants were placed in 12 patients.

Biopsies were immediately fixed in 10% buffered formaldehyde solution for 3 days and then processed, as described elsewhere (de Molon; de Paula; Spin-Neto; Verzola; Tosoni; Lia; Scaf & Marcantonio 2015). Serial sections of 6-μm thickness were obtained using an automatic microtome (Jung Supercut 2065, Leica Instruments GmbH, Heidelberg, Germany),

mounted on slides and stained with hematoxylin and eosin (H/E). The histological evaluation was made using an optical microscope (Diastar; Leica microsystems GmbH, Wetzlar, Germany) at 100× magnification. Images were selected and transferred to a computer display through a digital camera attached to the optical microscope (DFC-300-FX, Leica microsystems GmbH, Wetzlar, Germany) allowing histomorphometric analysis.

Two-blinded examiner performed the histomorphometric analysis. The digital images of histological slides were imported and analyzed into the program Image J for Windows (Image J 1.45; Wayne Rasband National Institutes of Health, USA). The histomorphometric analysis was made to measure the newly formed bone, the percentage of new bone formation, the reminiscent bone graft, and the amount of fibrous tissue 4 and 8 months after sinus floor augmentation. Selected slides for histomorphometric analysis followed the semi-series standard: the first section of the first slide was selected, and then four sections sequencing were despised and so on. Ten sections were evaluated from each specimen.

Resonance frequency analysis

The implant stability quotient (ISQ) was measured with a RFA device (Osstell; Integration Diagnostics, Gothenburg, Sweden). SmartPegs were used to measure the implant stability immediately after implant placement in both groups. The RFA device determines the resonance frequency of a peg, which can be attached to the implant with the aid of a cylindrical holder (de Molon et al. 2017, Dos Anjos; de Molon; Paim; Marcantonio; Marcantonio & Faeda 2016, Dursun et al. 2012). The Osstell apparatus makes contact-free measurements over a range of frequencies by exciting the SmartPeg, which starts to vibrate when the highest and lowest resonance frequencies occur. The measurements were performed in two directions, buccal-lingual and mesio-distal, and the mean values were used (Dos Anjos; de Molon; Paim; Marcantonio; Marcantonio & Faeda 2016). The ISQ value, displayed on the screen of the analyzer, ranges between 1 and 100; a high ISQ value indicates great stability

and a low value indicates a reduced integration between the implant and the surrounding bone. The ISQ measurements were performed in a standardized manner by one experienced, and calibrated examiner, who was masked to the treatment protocol.

Statistical analysis

Statistical analysis was performed using the GraphPad Prism software (version 6.0, GraphPad Software, Inc., La Jolla, CA, USA). All data were expressed as the mean ± the standard error of the mean (SEM). All data were submitted to the D'Agostino & Pearson test to assess the normality of data distribution. Wilcoxon test and paired t-test were used according to the data distribution. Differences were considered significant at $P < 0.05$.

RESULTS

Patient characteristics

Maxillary sinus floor augmentation was performed bilaterally in twelve patients (six were male and six were female), and they ranged in age from 43 to 63 years (with mean age of 54.17 ± 6.95 years). One side, randomly selected, was filled with DBBM + L-PRF and the contralateral side were grafted with DBBM only. Implants were installed after 4 and 8 months post-operative for the test and control group, respectively. During the healing period, the patients did not wear any provisional removable denture. After the healing period, 38 implants were installed, 19 in the control group, and 19 in the test group. No complications were observed during or after the sinus augmentation procedure. No perforation of the sinus membrane was verified, and none of the implants inserted was lost during the follow-up period. Additionally, no complications such as migration of graft material or opening of wound edges were observed in the test or control groups. To further minimize bias and simplify data analysis all implants installed were at the same widths and lengths

Cone-beam computed tomography analysis

To evaluate the repeatability of the measurements, Pearson correlation coefficient (r) was calculated in the differences of the three measurements in five samples with an r-value of 0.995 ($P<0.001$). A total of 36 CBCT scans were taken from 12 patients to evaluate bone dimensional changes after graft placement in the maxillary sinus (Fig. 1).

Immediately after graft placement (baseline) no differences were noted between both groups (1.69 ± 0.42 cm^3 for the test group and 1.46 ± 0.53 cm^3 for the control group) (Fig. 2A). Comparing the bone volume changes between test and control group after the healing period (4 and 8 months for the test and control group, respectively) no significant differences were noted between them (1.11 ± 0.25 cm^3 and 0.92 ± 0.35 cm^3 for test and control group, respectively) (Fig. 2B). The resorption rates between groups were also not statistically significant after the healing period ($33.13 \pm 10.74\%$ for the test and $36.71 \pm 15.81\%$ for the control group) (Fig 2C). After 4 months of healing, a decrease in bone volume was observed for the test group compared to the baseline ($P<0.0001$) (Fig. 2D). Similar data was noted comparing the control group at baseline and after 8 months ($P=0.0067$) (Fig. 2E).

Bone histomorphometry analysis

Immediately before implant placement, bone biopsies were gathered from the maxillary sinus in both sides for descriptive and histomorphometric analysis (Fig. 3). Our findings revealed statistically significant increase ($P=0.0083$) in the amount of newly formed bone between test (2.35 ± 0.73 mm^2) and control group (1.58 ± 0.44 mm^2) (Fig. 4A). Consequently, the percentage of new bone formation (Fig. 4B) was significantly increased ($P=0.0087$) in the test group ($44.58 \pm 13.89\%$) compared to the control group ($30.01 \pm 8.41\%$).

Significant increase ($P=0.0104$) in the amount of reminiscent graft material was found between control (0.71 ± 0.51 mm^2) and test (0.18 ± 0.22 mm^2) groups (Fig. 5A). As expected,

the percentage of bone graft (Fig. 5B) between groups was also significant increased (P=0.0111) in the control group (13.75 ± 9.99%) compared to the test group (3.59 ± 4.22%). Related to the amount of fibrous tissue (Fig. 6A) in the maxillary sinus cavities, the data demonstrated no significant differences between groups. The control group showed slight increase in the amount of fibrous tissue (1.60 ± 0.65 mm^2) compared to the test group (1.40 ± 0.58 mm^2). Similarly, the percentage of fibrous tissue (Fig. 6B) was also not different between groups (30.64 ± 12.46% and 26.60 ± 11.13% for the control and test groups, respectively).

Resonance frequency analysis

ISQ was measured immediately after implant placement, four and eight months after sinus augmentation for the test and control group, respectively. All implants evaluated in both groups demonstrated high ISQ values. The results showed a statistically increase (P=0.0003) in the ISQ values for the control group (75.13 ± 5.68) compared to the test group (60.90 ± 9.35) (Fig. 7).

DISCUSSION

The ideal time for implant placement is dependent of several aspects mainly related to the recipient area, socket dimension, bone quality and quantity, and time required for partial or complete tissue healing (Kotsakis et al. 2016). Autogenous bone, the gold standard graft material for sinus augmentation, possess several advantages because of its osteogenic, osteoinductive and osteoconductive properties (Dos Anjos; de Molon; Paim; Marcantonio; Marcantonio & Faeda 2016). However, the limited disponibility and the morbidity during graft removal are drawbacks related to this approach (Jensen & Terheyden 2009). Consequently, the use of biomaterials to fill the maxillary sinus is necessary. In this context, DBBM is a widely used biomaterial due to its similarity to the human bone, and higher rate of

clinical success (Hallman & Thor 2008). Nevertheless, DBBM lacks the osteogenic properties acting mainly as a scaffold for new bone formation. Importantly, the maturation of this type of material may take up to 8 months (Choukroun; Diss; Simonpieri; Girard; Schoeffler; Dohan; Dohan; Mouhyi & Dohan 2006) before implants can be placed in maxillary sinus, which might be considered a disadvantage of this material. To suppress this shortcoming, the addition of growth factors, particularly L-PRF, to the graft material has been suggested as an alternative approach to increase bone formation, enhance implant stability, favor osseointegration, and accelerate tissue maturation and healing (Ali; Bakry & Abd-Elhakam 2015, Bolukbasi; Ersanli; Keklikoglu; Basegmez & Ozdemir 2015, Boora; Rathee & Bhoria 2015, Castro; Meschi; Temmerman; Pinto; Lambrechts; Teughels & Quirynen 2017, Hallman & Thor 2008, Inchingolo et al. 2010, Jeong et al. 2014, Kotsakis; Boufidou; Hinrichs; Prasad; Rohrer & Tosios 2016, Marrelli & Tatullo 2013, Mazor et al. 2009, Nizam; Eren; Akcali & Donos 2017, Ocak; Kutuk; Demetoglu; Balcioglu; Ozdamar & Alkan 2017, Oncu & Alaaddinoglu 2015, Oncu; Bayram; Kantarci; Gulsever & Alaaddinoglu 2016, Oncu & Erbeyoglu 2017, Oncu & Kaymaz 2017).

In this study we aimed to better characterize the real effects of adding L-PRF to demineralized bovine graft material into the maxillary sinus. We have used different methodologies to evaluate bone volume alterations, bone characteristics and implant stability by means of CBCT, histology and RFA analyses. Our results demonstrated that there were no differences related to the bone volumetric changes between both groups immediately after graft placement and after the healing period of 4 and 8 months for the test and control group, respectively. However, a significant decrease in bone volume between periods (baseline and after the healing time) was observed for both groups. Interestingly, the amount of newly formed bone was significantly increased when L-PRF was added to the graft compared to the control group. As expected, the amount of reminiscent graft was significantly increased in the control group compared to the test, and no differences were observed between groups for the

amount of fibrous tissue. Finally, ISQ values were statistically significant higher in the control group compared to the test, however for both groups a safe ISQ values were found, which enabled implant osseointegration even after 4 months of healing for the L-PRF group.

CBCT has been considered a reliable technique for 3D visualization of the volumetric bone changes after sinus floor augmentation. Here, we have taken 3 different CBCTs at different times points: immediately after bone graft and after 4 and 8 months of healing. Our results did not reveal any statistical significant differences between both groups for volumetric bone alterations, according to figure 1. The only difference found was observed between periods for the test and control group where both groups showed decreased bone volume after the healing period. These variations in the volumetric changes were also observed in previous studies (Kirmeier et al. 2008, Klein et al. 2016, Kuhl et al. 2014). The variations found in the literature in regard to the dimensional changes could be influenced by the reminiscent alveolar ridge, maxillary sinus anatomy, amount of grafted material into the sinus, and compression force during graft placement (Gultekin et al. 2016, Kirmeier; Payer; Wehrschuetz; Jakse; Platzer & Lorenzoni 2008, Shanbhag et al. 2014). Our group recently demonstrated that the mixture of L-PRF to the DBBM for sinus augmentation lead to a decrease in bone volume for the sinus grafted with the L-PRF after 8 months of healing. We claimed that the lower amount of DBBM inserted in the test group, the surgical technique, and the L-PRF degradation over time might be accounted for the lower volume in the sinus. Accordingly, previous studies demonstrated that the mean volume reduction of DBBM in maxillary sinus ranged from 15.2 to 21.5% (Kuhl; Payer; Kirmeier; Wildburger; Wegscheider & Jakse 2014).

The maturation time for implant placement after sinus augmentation with Bio-Oss® may take several weeks to form new bone, closely to 8 months of healing for a safe implant installation (Choukroun; Diss; Simonpieri; Girard; Schoeffler; Dohan; Dohan; Mouhyi & Dohan 2006, Martinez et al. 2001). This fact could be closely related, despite of the graft

characteristics, to the graft volume. Consequently, as higher the DBBM graft volume is higher is the time necessary before implants loading (Xuan et al. 2014). In the current study, the amount of DBBM was reduced in the test group since L-PRF was added to the graft material. This result could also be observed in Figure 3A-B, where the area (0.71 ± 0.51 mm^2 control group; 0.18 ± 0.22 mm^2 test group) and percentage (13.75 ± 9.99% control group; and 3.59 ± 4.22% for test group) of graft material present was statistically higher in the control compared to the test group. Furthermore, L-PRF contain dense fibrin fiber network that helps to avoid the small particles of DBBM from dispersing. This means that less amount of graft material is needed to fill the maxillary sinus to obtain a sufficient vertical bone height for appropriate implant length installation (Xuan; Lee; Son; Jeong & Choi 2014). Moreover, the fibrin fiber presents a promising impact on handling and adhesion to the bone defect walls (Le Guehennec et al. 2004). Paralleling recent observations (Xuan; Lee; Son; Jeong & Choi 2014), our findings indicate that L-PRF can act as a delivery system for DBBM particles during sinus lifting.

To investigate the implant stability, RFA analysis was performed immediately after implant placement for both groups through measurement of the ISQ as a function of stiffness of the bone-implant interface (de Molon; Lages; Rivera; de Souza Faloni; Margonar & Queiroz 2017, Dos Anjos; de Molon; Paim; Marcantonio; Marcantonio & Faeda 2016). This measurement is affected by innumerous factors, such as the healing time, bone quality and density, firmness of the fixation, degree of osseointegration, hardness of the bone, and the implant height above the alveolar crest (Sennerby et al. 2015, Sennerby & Meredith 2008, Sennerby et al. 2015). It was previously pointed out that ISQ values ranging from 57 to 82 denote appropriate implant stability and a complete process of osseointegration (Balleri et al. 2002). In the current study, the control group showed statistically higher ISQ values compared to the control group (75.13 ± 5.68; and 60.90 ± 9.35 for the control and test group, respectively). This outcome could be attributed to the difference in the healing time between

both groups. According to a previous study (Tabrizi; Arabion & Karagah 2017), ISQ values after sinus augmentation utilizing L-PRF progressively increase over time, meaning that the time for implant healing play a crucial role for increased secondary implant stability. Recent observations demonstrated that the addition of L-PRF improved implant stability and delivered faster osseointegration (Oncu & Alaaddinoglu 2015). The difference between our studies and their study might be accounted for the implant sites (posterior vs anterior), differences in bone quality, and healing period (baseline, 1 and 4 weeks of healing). The present data suggest that a site undergoing sinus augmentation with DBBM + L-PRF can offer sufficient implant stability, decreasing the necessary time for bone graft maturation allowing earlier implant placement.

An important consideration should be mention when interpreting the present outcomes. The limitation of this investigation is that the groups (test and control) were compared to a different time points (8 months for the control and 4 months for the test group). With this experimental design, it is not possible to confirm if there would be more new bone on the control group if we had gone in earlier to place the implants. Furthermore, ISQ values would probably increase in the test group if measurements were taken after 8 months of healing. All of these concerns might biases the results, however, our data pointed out to important aspects (especially the histology data) encouraging earlier intervention in the maxillary sinus grafted with DBBM + L-PRF.

CONCLUSION

Our data demonstrated that the addition of L-PRF to the DBBM graft increased the newly formed bone after 4 months of healing. The residual graft material was statistically lower in the test group, which might have influenced the early maturation of the bone graft. No volumetric dimensional changes were observed between both groups in the periods evaluated. Furthermore, ISQ values were sufficiently higher to allow implant osseointegration for both

groups. Taken together, our data suggest that L-PRF lead to faster bone graft maturation allowing earlier implant placement in the conditions studied.

REFERENCES

Ali, S., Bakry, S. A. & Abd-Elhakam, H. (2015) Platelet-rich fibrin in maxillary sinus augmentation: A systematic review. *The Journal of oral implantology* **41**: 746-753.

Angelo, T., Marcel, W., Andreas, K. & Izabela, S. (2015) Biomechanical stability of dental implants in augmented maxillary sites: Results of a randomized clinical study with four different biomaterials and prf and a biological view on guided bone regeneration. *BioMed research international* **2015**: 850340.

Balleri, P., Cozzolino, A., Ghelli, L., Momicchioli, G. & Varriale, A. (2002) Stability measurements of osseointegrated implants using osstell in partially edentulous jaws after 1 year of loading: A pilot study. *Clin Implant Dent Relat Res* **4**: 128-132.

Bolukbasi, N., Ersanli, S., Keklikoglu, N., Basegmez, C. & Ozdemir, T. (2015) Sinus augmentation with platelet-rich fibrin in combination with bovine bone graft versus bovine bone graft in combination with collagen membrane. *The Journal of oral implantology* **41**: 586-595.

Boora, P., Rathee, M. & Bhoria, M. (2015) Effect of platelet rich fibrin (prf) on peri-implant soft tissue and crestal bone in one-stage implant placement: A randomized controlled trial. *Journal of clinical and diagnostic research : JCDR* **9**: ZC18-21.

Boyne, P. J. & James, R. A. (1980) Grafting of the maxillary sinus floor with autogenous marrow and bone. *J Oral Surg* **38**: 613-616.

Browaeys, H., Bouvry, P. & De Bruyn, H. (2007) A literature review on biomaterials in sinus augmentation procedures. *Clin Implant Dent Relat Res* **9**: 166-177.

Castro, A. B., Meschi, N., Temmerman, A., Pinto, N., Lambrechts, P., Teughels, W. & Quirynen, M. (2017) Regenerative potential of leucocyte- and platelet-rich fibrin. Part b: Sinus floor elevation, alveolar ridge preservation and implant therapy. A systematic review. *J Clin Periodontol* **44**: 225-234.

Chang, I. C., Tsai, C. H. & Chang, Y. C. (2010) Platelet-rich fibrin modulates the expression of extracellular signal-regulated protein kinase and osteoprotegerin in human osteoblasts. *J Biomed Mater Res A* **95**: 327-332.

Chiapasco, M., Casentini, P. & Zaniboni, M. (2009) Bone augmentation procedures in implant dentistry. *The International journal of oral & maxillofacial implants* **24 Suppl**: 237-259.

Choukroun, J., Diss, A., Simonpieri, A., Girard, M. O., Schoeffler, C., Dohan, S. L., Dohan, A. J., Mouhyi, J. & Dohan, D. M. (2006) Platelet-rich fibrin (prf): A second-generation platelet concentrate. Part v: Histologic evaluations of prf effects on bone allograft maturation in sinus lift. *Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics* **101**: 299-303.

Cordaro, L., Torsello, F., Accorsi Ribeiro, C., Liberatore, M. & Mirisola di Torresanto, V. (2010) Inlay-onlay grafting for three-dimensional reconstruction of the posterior atrophic maxilla with mandibular bone. *Int J Oral Maxillofac Surg* **39**: 350-357.

Davis, V. L., Abukabda, A. B., Radio, N. M., Witt-Enderby, P. A., Clafshenkel, W. P., Cairone, J. V. & Rutkowski, J. L. (2014) Platelet-rich preparations to improve healing. Part i: Workable options for every size practice. *The Journal of oral implantology* **40**: 500-510.

Davis, V. L., Abukabda, A. B., Radio, N. M., Witt-Enderby, P. A., Clafshenkel, W. P., Cairone, J. V. & Rutkowski, J. L. (2014) Platelet-rich preparations to improve healing. Part ii: Platelet activation and enrichment, leukocyte inclusion, and other selection criteria. *The Journal of oral implantology* **40**: 511-521.

de Molon, R. S., de Paula, W. N., Spin-Neto, R., Verzola, M. H., Tosoni, G. M., Lia, R. C., Scaf, G. & Marcantonio, E., Jr. (2015) Correlation of fractal dimension with histomorphometry in maxillary sinus lifting using autogenous bone graft. *Braz Dent J* **26**: 11-18.

de Molon, R. S., Lages, F. S., Rivera, C. P., de Souza Faloni, A. P., Margonar, R. & Queiroz, T. P. (2017) Evaluation of short and regular implants after prosthesis placement in the mandible: A nonrandomized controlled clinical trial. *The journal of contemporary dental practice* **18**: 1122-1129.

de Molon, R. S., Morais-Camilo, J. A., Verzola, M. H., Faeda, R. S., Pepato, M. T. & Marcantonio, E., Jr. (2013) Impact of diabetes mellitus and metabolic control on bone healing around osseointegrated implants: Removal torque and histomorphometric analysis in rats. *Clinical oral implants research* **24**: 831-837.

Del Corso, M., Mazor, Z., Rutkowski, J. L. & Dohan Ehrenfest, D. M. (2012) The use of leukocyte- and platelet-rich fibrin during immediate postextractive implantation and loading for the esthetic replacement of a fractured maxillary central incisor. *The Journal of oral implantology* **38**: 181-187.

Dohan, D. M., Choukroun, J., Diss, A., Dohan, S. L., Dohan, A. J., Mouhyi, J. & Gogly, B. (2006) Platelet-rich fibrin (prf): A second-generation platelet concentrate. Part i: Technological concepts and evolution. *Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics* **101**: e37-44.

Dos Anjos, T. L., de Molon, R. S., Paim, P. R., Marcantonio, E., Marcantonio, E., Jr. & Faeda, R. S. (2016) Implant stability after sinus floor augmentation with deproteinized bovine bone mineral particles of different sizes: A prospective, randomized and controlled split-mouth clinical trial. *Int J Oral Maxillofac Surg* **45**: 1556-1563.

Dursun, E., Tulunoglu, I., Canpinar, P., Uysal, S., Akalin, F. A. & Tozum, T. F. (2012) Are marginal bone levels and implant stability/mobility affected by single-stage platform switched dental implants? A comparative clinical study. *Clin Oral Implants Res* **23**: 1161-1167.

Faul, F., Erdfelder, E., Buchner, A. & Lang, A. G. (2009) Statistical power analyses using g*power 3.1: Tests for correlation and regression analyses. *Behav Res Methods* **41**: 1149-1160.

Gultekin, B. A., Cansiz, E., Borahan, O., Mangano, C., Kolerman, R., Mijiritsky, E. & Yalcin, S. (2016) Evaluation of volumetric changes of augmented maxillary sinus with different bone grafting biomaterials. *The Journal of craniofacial surgery* **27**: e144-148.

Hallman, M. & Thor, A. (2008) Bone substitutes and growth factors as an alternative/complement to autogenous bone for grafting in implant dentistry. *Periodontology 2000* **47**: 172-192.

Harris, D., Horner, K., Grondahl, K., Jacobs, R., Helmrot, E., Benic, G. I., Bornstein, M. M., Dawood, A. & Quirynen, M. (2012) E.A.O. Guidelines for the use of diagnostic imaging in implant dentistry 2011. A consensus workshop organized by the european association for osseointegration at the medical university of warsaw. *Clin Oral Implants Res* **23**: 1243-1253.

Inchingolo, F., Tatullo, M., Marrelli, M., Inchingolo, A. M., Scacco, S., Inchingolo, A. D., Dipalma, G., Vermesan, D., Abbinante, A. & Cagiano, R. (2010) Trial with platelet-rich fibrin and bio-oss used as grafting materials in the treatment of the severe maxillar bone atrophy: Clinical and radiological evaluations. *European review for medical and pharmacological sciences* **14**: 1075-1084.

Jensen, S. S. & Terheyden, H. (2009) Bone augmentation procedures in localized defects in the alveolar ridge: Clinical results with different bone grafts and bone-substitute materials. *The International journal of oral & maxillofacial implants* **24 Suppl**: 218-236.

Jeong, S. M., Lee, C. U., Son, J. S., Oh, J. H., Fang, Y. & Choi, B. H. (2014) Simultaneous sinus lift and implantation using platelet-rich fibrin as sole grafting material. *J Craniomaxillofac Surg* **42**: 990-994.

Jung, R. E., Schmoekel, H. G., Zwahlen, R., Kokovic, V., Hammerle, C. H. & Weber, F. E. (2005) Platelet-rich plasma and fibrin as delivery systems for recombinant human bone morphogenetic protein-2. *Clin Oral Implants Res* **16**: 676-682.

Kang, Y. H., Jeon, S. H., Park, J. Y., Chung, J. H., Choung, Y. H., Choung, H. W., Kim, E. S. & Choung, P. H. (2011) Platelet-rich fibrin is a bioscaffold and reservoir of growth factors for tissue regeneration. *Tissue engineering. Part A* **17**: 349-359.

Kazemi, E., Dadfarnia, S., Haji Shabani, A. M. & Ranjbar, M. (2017) Synthesis, characterization, and application of a zn (ii)-imprinted polymer grafted on graphene oxide/magnetic chitosan nanocomposite for selective extraction of zinc ions from different food samples. *Food chemistry* **237**: 921-928.

Kirmeier, R., Payer, M., Wehrschuetz, M., Jakse, N., Platzer, S. & Lorenzoni, M. (2008) Evaluation of three-dimensional changes after sinus floor augmentation with different grafting materials. *Clin Oral Implants Res* **19**: 366-372.

Klein, G. G., Curvello, V. P., Dutra, R. A., Simeao, S. P., Santos, P. L., Gulinelli, J. L. & Filho, H. N. (2016) Bone volume changes after sinus floor augmentation with heterogenous graft. *The International journal of oral & maxillofacial implants* **31**: 665-671.

Kotsakis, G. A., Boufidou, F., Hinrichs, J. E., Prasad, H. S., Rohrer, M. & Tosios, K. I. (2016) Extraction socket management utilizing platelet rich fibrin: A proof-of-principle study of the "accelerated-early implant placement" concept. *The Journal of oral implantology* **42**: 164-168.

Kuhl, S., Payer, M., Kirmeier, R., Wildburger, A., Wegscheider, W. & Jakse, N. (2014) The influence of bone marrow aspirates and concentrates on the early volume stability of

maxillary sinus grafts with deproteinized bovine bone mineral - first results of a rct. *Clin Oral Implants Res* **25**: 221-225.

Kumar, R. V. & Shubhashini, N. (2013) Platelet rich fibrin: A new paradigm in periodontal regeneration. *Cell and tissue banking* **14**: 453-463.

Le Guehennec, L., Layrolle, P. & Daculsi, G. (2004) A review of bioceramics and fibrin sealant. *European cells & materials* **8**: 1-10; discussion 10-11.

Marrelli, M. & Tatullo, M. (2013) Influence of prf in the healing of bone and gingival tissues. Clinical and histological evaluations. *European review for medical and pharmacological sciences* **17**: 1958-1962.

Martinez, H., Davarpanah, M., Missika, P., Celletti, R. & Lazzara, R. (2001) Optimal implant stabilization in low density bone. *Clin Oral Implants Res* **12**: 423-432.

Marx, R. E., Carlson, E. R., Eichstaedt, R. M., Schimmele, S. R., Strauss, J. E. & Georgeff, K. R. (1998) Platelet-rich plasma: Growth factor enhancement for bone grafts. *Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics* **85**: 638-646.

Mazor, Z., Horowitz, R. A., Del Corso, M., Prasad, H. S., Rohrer, M. D. & Dohan Ehrenfest, D. M. (2009) Sinus floor augmentation with simultaneous implant placement using choukroun's platelet-rich fibrin as the sole grafting material: A radiologic and histologic study at 6 months. *Journal of periodontology* **80**: 2056-2064.

Nizam, N., Eren, G., Akcali, A. & Donos, N. (2017) Maxillary sinus augmentation with leukocyte and platelet-rich fibrin and deproteinized bovine bone mineral: A split-mouth histological and histomorphometric study. *Clin Oral Implants Res*.

Ocak, H., Kutuk, N., Demetoglu, U., Balcioglu, E., Ozdamar, S. & Alkan, A. (2017) Comparison of bovine bone-autogenic bone mixture versus platelet-rich fibrin for maxillary sinus grafting: Histologic and histomorphologic study. *The Journal of oral implantology* **43**: 194-201.

Oncu, E. & Alaaddinoglu, E. E. (2015) The effect of platelet-rich fibrin on implant stability. *The International journal of oral & maxillofacial implants* **30**: 578-582.

Oncu, E., Bayram, B., Kantarci, A., Gulsever, S. & Alaaddinoglu, E. E. (2016) Positive effect of platelet rich fibrin on osseointegration. *Medicina oral, patologia oral y cirugia bucal* **21**: e601-607.

Oncu, E. & Erbeyoglu, A. A. (2017) Enhancement of immediate implant stability and recovery using platelet-rich fibrin. *The International journal of periodontics & restorative dentistry*.

Oncu, E. & Kaymaz, E. (2017) Assessment of the effectiveness of platelet rich fibrin in the treatment of schneiderian membrane perforation. *Clin Implant Dent Relat Res* **19**: 1009-1014.

Pelo, S., Gasparini, G., Moro, A., Boniello, R. & Amoroso, P. F. (2009) Segmental le fort i osteotomy with bone grafting in unilateral severely atrophied maxilla. *Int J Oral Maxillofac Surg* **38**: 246-249.

Scarano, A., Piattelli, A., Perrotti, V., Manzon, L. & Iezzi, G. (2011) Maxillary sinus augmentation in humans using cortical porcine bone: A histological and histomorphometrical evaluation after 4 and 6 months. *Clin Implant Dent Relat Res* **13**: 13-18.

Schulz, K. F., Altman, D. G., Moher, D. & Group, C. (2010) Consort 2010 statement: Updated guidelines for reporting parallel group randomised trials. *BMC Med* **8**: 18.

Sennerby, L., Andersson, P., Pagliani, L., Giani, C., Moretti, G., Molinari, M. & Motroni, A. (2015) Evaluation of a novel cone beam computed tomography scanner for bone density examinations in preoperative 3d reconstructions and correlation with primary implant stability. *Clin Implant Dent Relat Res* **17**: 844-853.

Sennerby, L. & Meredith, N. (2008) Implant stability measurements using resonance frequency analysis: Biological and biomechanical aspects and clinical implications. *Periodontology 2000* **47**: 51-66.

Sennerby, L., Pagliani, L., Petersson, A., Verrocchi, D., Volpe, S. & Andersson, P. (2015) Two different implant designs and impact of related drilling protocols on primary stability in different bone densities: An in vitro comparison study. *The International journal of oral & maxillofacial implants* **30**: 564-568.

Shanbhag, S., Shanbhag, V. & Stavropoulos, A. (2014) Volume changes of maxillary sinus augmentations over time: A systematic review. *The International journal of oral & maxillofacial implants* **29**: 881-892.

Tabrizi, R., Arabion, H. & Karagah, T. (2017) Does platelet-rich fibrin increase the stability of implants in the posterior of the maxilla? A split-mouth randomized clinical trial. *Int J Oral Maxillofac Surg*.

Tadjoedin, E. S., de Lange, G. L., Bronckers, A. L., Lyaruu, D. M. & Burger, E. H. (2003) Deproteinized cancellous bovine bone (bio-oss) as bone substitute for sinus floor elevation. A retrospective, histomorphometrical study of five cases. *J Clin Periodontol* **30**: 261-270.

Tajima, N., Ohba, S., Sawase, T. & Asahina, I. (2013) Evaluation of sinus floor augmentation with simultaneous implant placement using platelet-rich fibrin as sole grafting material. *The International journal of oral & maxillofacial implants* **28**: 77-83.

Tatum, H., Jr. (1986) Maxillary and sinus implant reconstructions. *Dental clinics of North America* **30**: 207-229.

Tetsch, J., Tetsch, P. & Lysek, D. A. (2010) Long-term results after lateral and osteotome technique sinus floor elevation: A retrospective analysis of 2190 implants over a time period of 15 years. *Clin Oral Implants Res* **21**: 497-503.

Xuan, F., Lee, C. U., Son, J. S., Jeong, S. M. & Choi, B. H. (2014) A comparative study of the regenerative effect of sinus bone grafting with platelet-rich fibrin-mixed bio-oss(r) and commercial fibrin-mixed bio-oss(r): An experimental study. *J Craniomaxillofac Surg* **42**: e47-50.

FIGURE LEGENDS

Figure 1. Representative CBCT images from Planmeca software. A) Pre-operative scan showing residual bone in posterior maxilla. B) Post-operative analyses from grafted sinus mensured in cm^3.

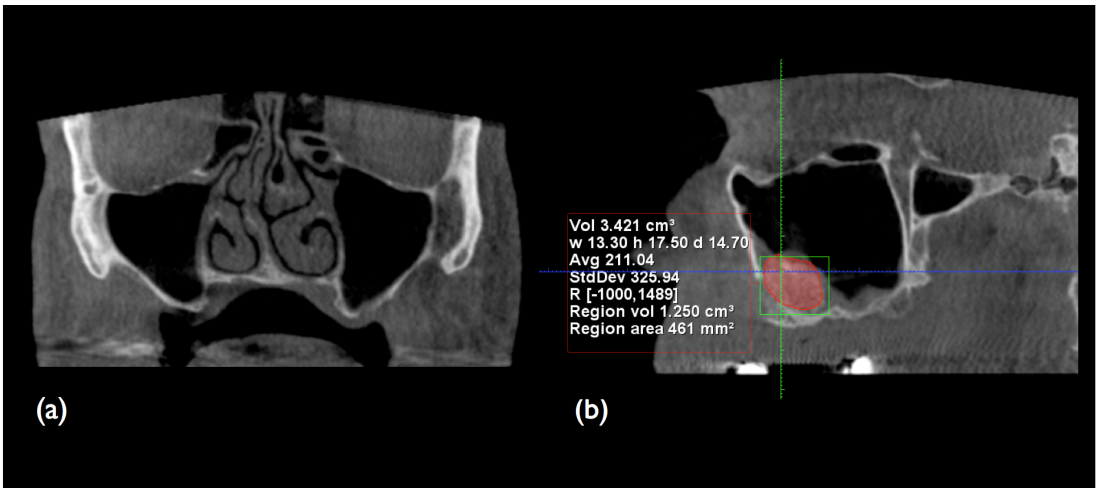


Figure 2. Bone volumetric dimensional changes measured by means of CBCT in both groups. A) Bone volume measured at baseline before maxillary sinus augmentation. B) Bone volumetric changes after 4 and 8 months of healing for control (Bio-Oss) and test (Bio-Oss + L-PRF). C) Percentage of volumetric changes between groups after the healing time. D) Volumetric changes in the test group after the healing period of 4 months. E) Volumetric changes after 8-months of healing for the control group. **Statistically significant difference from indicated group, $p<0.005$. ***Statistically significant difference from indicated group, $p<0.0001$. Differences between groups were calculated by paired and unpaired t-test. Data represent the mean ± SEM.

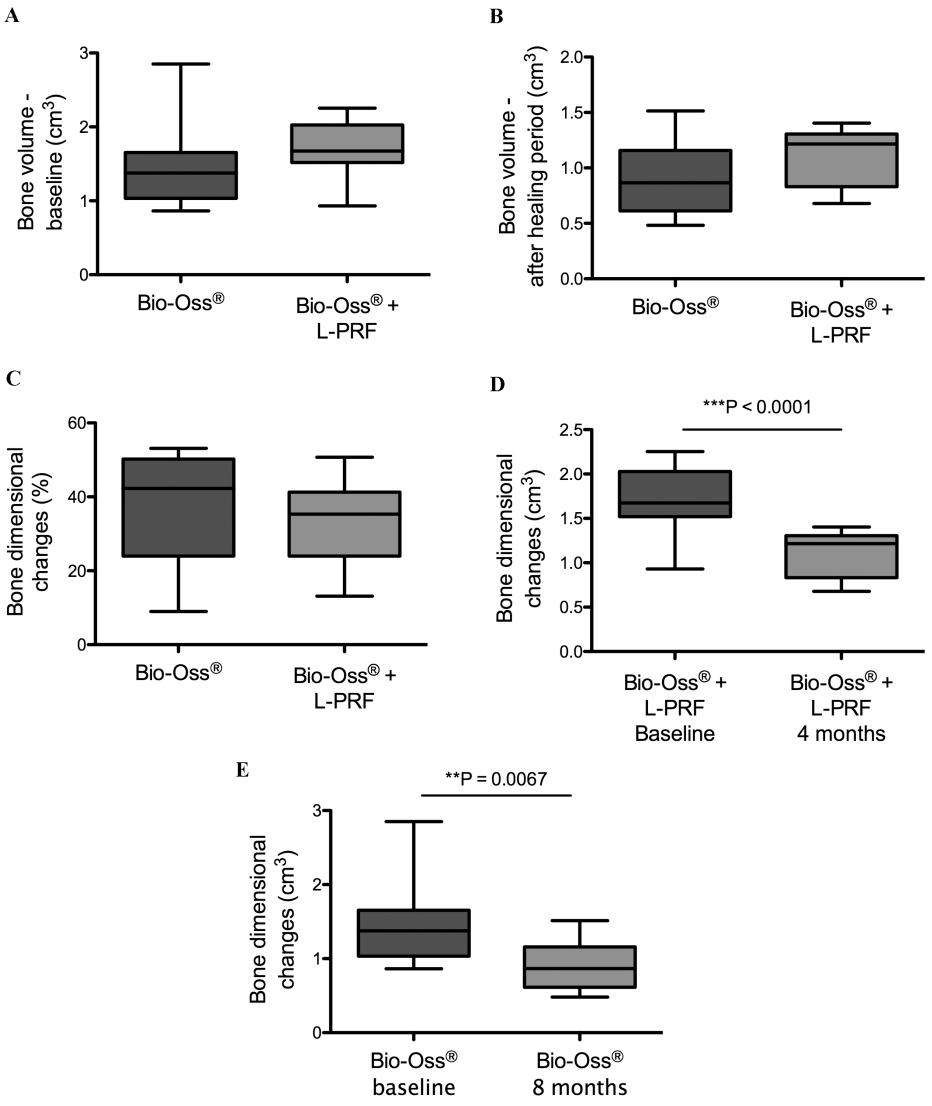


Figure 3. A) Representative histological section from group BioOss + L-PRFand **B)** only Bio-Oss. NB is native bone; NFL corresponds to the newly formed bone; B is biomaterial; and ST is soft tissue. Yellow color corresponds to the newly formed bone; red is biomaterial; Blue is native bone.

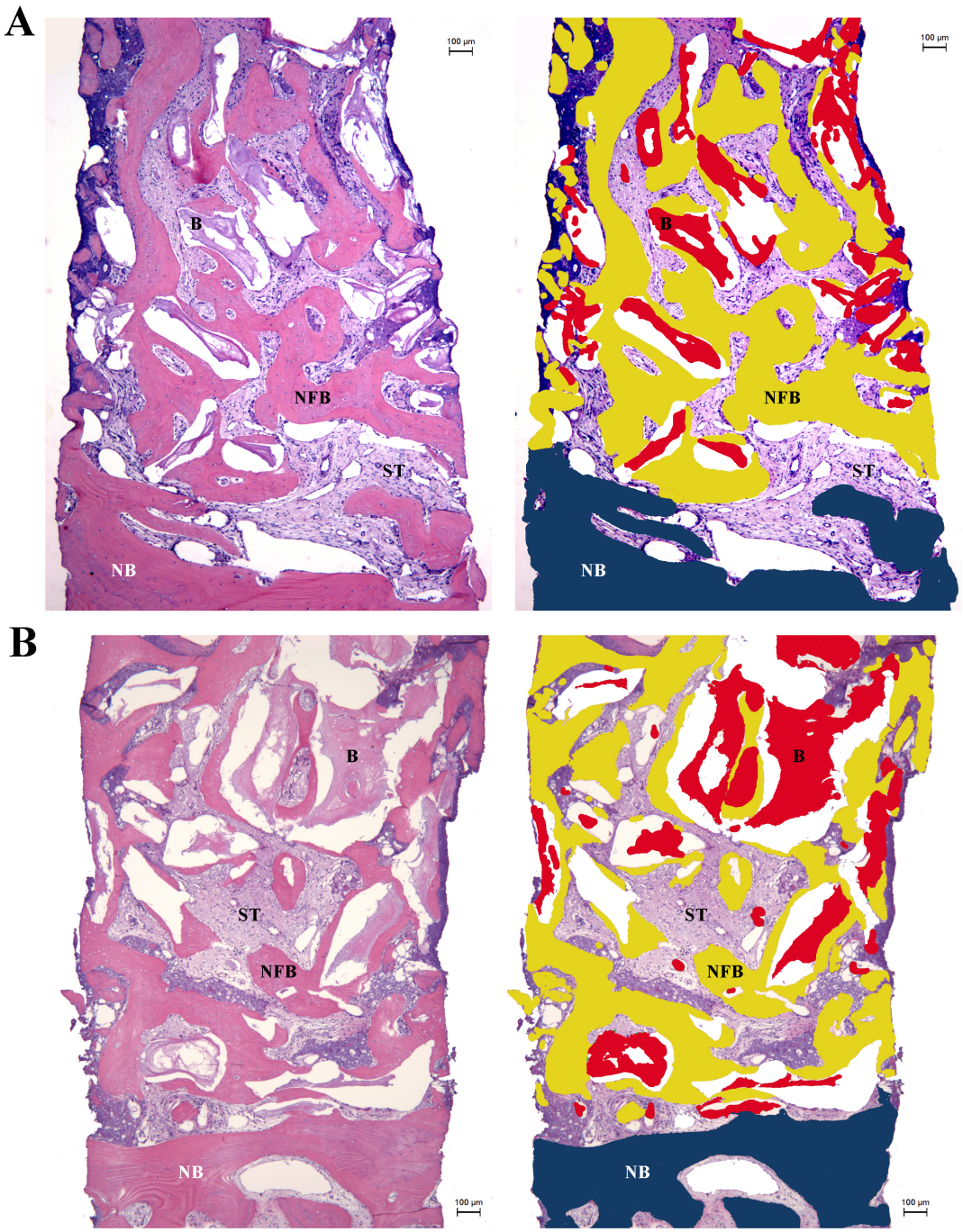


Figure 4. Histomorphometric analysis evaluating A) newly formed bone (mm^2) and B) percentage of new bone formation between groups. **Statistically significant difference from indicated group, $p<0.05$. Differences between groups were calculated by paired and unpaired t-test. Data represent the mean ± SEM.

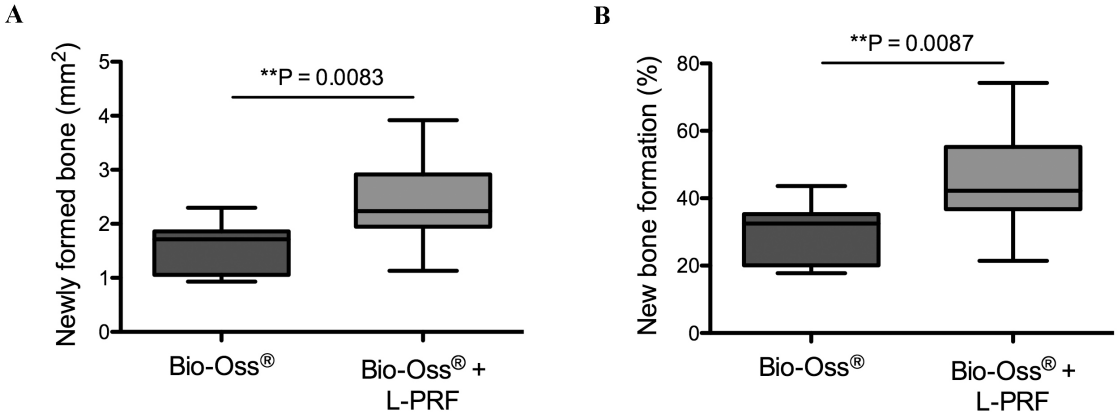


Figure 5. Histomorphometric analysis evaluating A) area and B) percentage of residual bone graft material between groups. *Statistically significant difference from indicated group, $p<0.01$. Differences between groups were calculated by paired and unpaired t-test. Data represent the mean ± SEM.

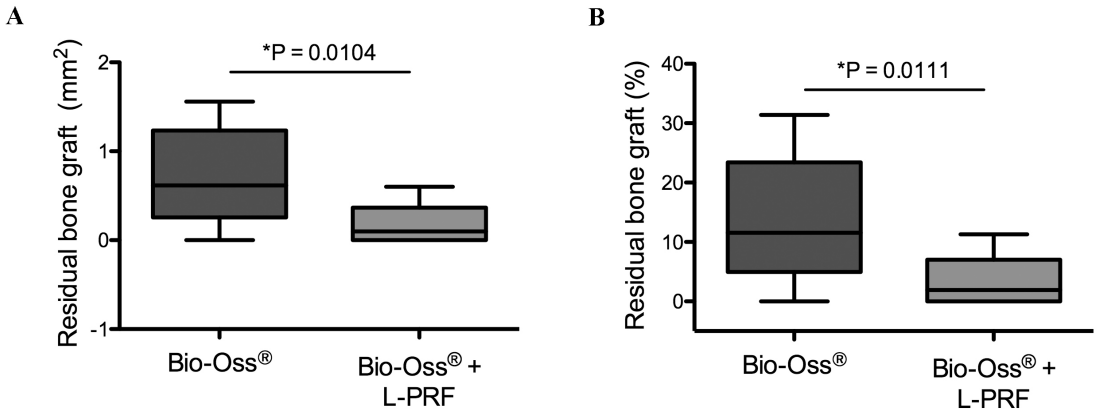


Figure 6. Histomorphometric analysis evaluating A) area and B) percentage of soft (fibrous) tissue in the maxillary sinus after the healing period. No differences were found for the amount of fibrous tissue. Differences between groups were calculated by paired and unpaired t-test. Data represent the mean ± SEM.

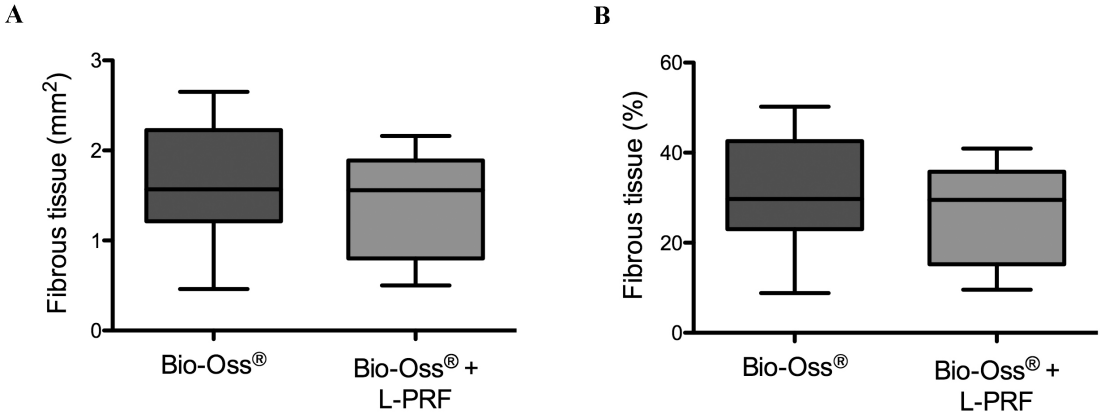
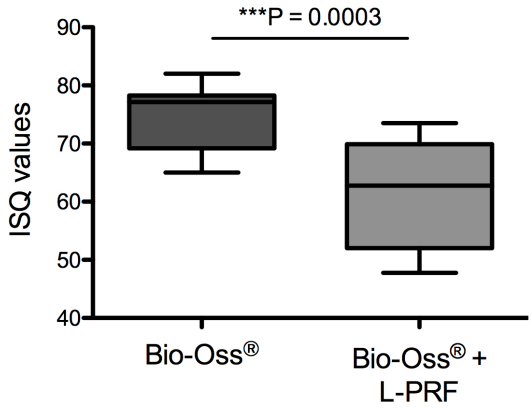


Figure 7. Implant stability quotient (ISQ) measured by means of resonance frequency analysis immediately after implant placement in both groups. ***Statistically significant difference from indicated group, p<0.0003. Differences between groups were calculated by paired and unpaired t-test. Data represent the mean ± SEM.



3.4 Publicação 4

Early placement of dental implants in maxillary sinus grafted with leukocyte and platelet-rich fibrin (L-PRF) and deproteinized bovine bone mineral[§]

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ABSTRACT

This case report aimed to describe the effects of leukocyte and platelet-rich fibrin (L-PRF) associated with deproteinized bovine bone mineral (DBBM) and absorbable collagen membrane (CM) on bone regeneration in maxillary sinus augmentation. A 59-year-old male patient was referred to the Department of Periodontology for implant rehabilitation of his edentulous upper jaw. The treatment plan involved maxillary sinus augmentation followed by implants installation. A split-mouth design was employed, in which the right maxillary sinus was filled using L-PRF, DBBM, and CM; the left side was filled with DBBM and CM. After four and eight-months post-operatively, two dental implants were installed in each of the right and left maxillary sinus, respectively. Cone-beam computed tomography (CBCT) was taken before and after sinus augmentation for evaluation of tridimensional bone volume alterations. Bone biopsies were harvested from the implant sites for histomorphometric evaluation. Resonance frequency analysis was employed immediately after implant placement and before prosthetic rehabilitation for evaluation of implant stability. Implants were loaded 10-months after sinus augmentation. CBCT analysis showed a higher resorption rate in the right side of the maxillary sinus (L-PRF + DBBM) compared to the left side (22.25% and 8,95%, respectively). Implant stability quotient were above 68 in all time-points for both groups. Histomorphometric analysis showed highly amount of newly formed bone when L-PRF was used compared with DBBM alone (2118102 and 975535 mm^3, respectively). Taken together, both techniques were effective for maxillary sinus augmentation, however the addition of L-PRF to the graft allowed early implant placement and accelerated bone healing in the conditions studied.

Key Words: Alveolar bone; cone-beam computed tomography; platelet-rich plasma; sinus floor augmentation; dental implants.

INTRODUCTION

Sinus lift, a surgical approach that enables the placement of appropriate length implants,[1] is achieved by means of the elevation of the sinus membrane allowing the interposition of bone grafts for the long term implant stability. DBBM (Bio-Oss® Geistlich Pharma AB) is an osteoconductive material, chemically and physically similar to human bone, that act as a scaffold allowing osteogenic cell transportation from the sinus wall to the graft particles increasing the potential of new bone formation.[2, 3] In order to enhance bone formation and accelerate bone healing, the addition of L-PRF has been proposed.[2, 4-9] Platelets present beneficial effects on tissue healing by means of release of growth factors, such as platelet-derived growth factor (PGDF), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF) and transforming growth factor (TGF-β).[5] The unique fibrin form can also promote angiogenesis and osteogenesis,[5] which might help to improve the regeneration of sinus cavities.[8] PRF can also modulate the expression of osteoprotegerin increasing osteoblast differentiation.[10] Furthermore, previous study demonstrated that L-PRF can be considered as an alternative material for repairing sinus perforations,[11] and socket management.[12] A recent systematic review[13] showed promising results when L-PRF was used as a sole filling material, and combined with bone substitutes into the maxillary sinus. Additionally, accelerated maturation of demineralized dried bone was evidenced.[13] Due to these characteristics, L-PRF has been accepted by the profession and is routinely used in the clinic.[14]

Although Bio-Oss®, the most utilized DBBM biomaterial, has often been used as a bone substitute,[15, 16] a small number of studies have addressed the effect of adding L-PRF for maxillary sinus augmentation in combination with DBBM. Moreover, there appear to be no studies that have utilized CBCT and RFA analyses to measure bone volume alterations and implant stability after sinus augmentation with L-PRF. Thus, the aim of this case was to report the effects of L-PRF associated with DBBM and CM on bone regeneration in maxillary

sinus augmentation, and the capability of L-PRF in accelerates bone healing for early implant placement.

CASE PRESENTATION

A 59-year-old male patient was referred to the Department of Periodontology for implant rehabilitation of his edentulous upper jaw (Fig. 1A). He had no relevant medical history that could compromise bone healing[17, 18] and denied smoking or the use of alcohol. A CBCT scan (iCat Classic; Imaging Sciences International - Hatfield, PA, USA) was rendered to evaluate the reminiscent vertical bone height (Fig. 1B). Given the limited amount of bone height in the posterior area of the maxilla, the proposed treatment plan involved maxillary sinus augmentation followed by implant installation. A split-mouth design was planned, in which the right maxillary sinus floor was augmented using leukocyte platelet-rich fibrin (L-PRF) associated with deproteinized bovine bone mineral (DBBM) (Bio-Oss; Geistlich Pharma AG, Wolhusen, Switzerland) and absorbable collagen membrane (CM) (Bio-Guide; Geistlich Pharma AG, Wolhusen, Switzerland) followed by implant placement after 4 months post-surgery; the left side (control side) was filled with DBBM and CM, and dental implants were installed after 8 months post-sinus augmentation. The patient was informed about the treatment and its possible side effects. Recommended treatment plan was accepted by the patient and written informed consent was signed. The study protocol was approved by the institutional ethics committee on human research (protocol number 41357514.5.0000.5416).

CASE MANAGEMENT

The addition of L-PRF to the bone graft in the right maxillary sinus was aimed to investigate the capability of this growth factor in accelerate bone healing for early implant placement, and to compare with the well established protocol using only DBBM in the left sinus. Prior to the maxillary sinus augmentation procedure, L-PRF was prepared according to previous

protocols.[6, 8, 19, 20] Briefly, peripheral blood sample was taken before the surgery, and immediately centrifuged at 300 x g (3000 RPM) for 10 min using an appropriate centrifuge (Kasvi K14-0815, Curitiba – Brazil). After centrifugation, the fibrin clot was removed from the tube and separated. The L-PRF clot was prepared in the form of a membrane by pressing out the fluids (Fig. 2A).[21] Subsequently, the L-PRF was mixed with the DBBM (0.25-1 mm particle sizes) to fill the right sinus (Fig 2B) in 1:3 proportions.

The sinus floor augmentation procedure was performed under local anesthesia (Articaine 4% and epinephrine 1:100,000; DFL, Rio de Janeiro, RJ, Brazil). A crestal incision was made in the maxillary edentulous area, followed by two vertical incisions extending both mesial and distal to the lateral sinus wall, as described previously.[22, 23] A lateral window approach was accomplished according to the technique first described by Boyne and James[1] (Fig. 3A). The mixture of L-PRF and DBBM and the DBBM only were inserted into the respective sinus cavities (Fig. 3B-C) and an absorbable collagen membrane was applied to cover the entire obturated lateral window (Fig. 3D). The flap was then repositioned and sutured with a nylon thread (Nylon 4-0; Ethicon, Jonhson & Jonhson S.A. Sao Paulo, SP, Brazil) to achieve primary wound closure.

After surgery, the patient received postoperative instructions for appropriate oral hygiene control, oral antibiotics (amoxicillin, 500 mg three times a day for a week), oral anti-inflammatory (nimesulide, 100 mg twice a day for 5 days), and analgesic (paracetamol, 750 mg every 6 hours for 2 days). He was advised to rinse his mouth with chlorhexidine (0.2%) for 14 days. The sutures were removed 10 days after the surgical procedure, and the area was not subjected to any direct loading during the entire bone regeneration phase.

Four-months after the sinus augmentation procedure, a new CBCT was taken to evaluate the achieved bone volume (Fig. 4A). Based on the tridimensional reconstruction (Fig. 4B), two dental implants (TitamaxTi EX Acqua 4 x 11 mm; external exagon, Neodent, Curitiba, PR, Brazil) were planned and installed in the right maxillary sinus and another six

implants (TitamaxTi EX Acqua 4 x 11 mm; internal exagon, Neodent, Curitiba, PR, Brazil) were also installed in the upper jaw except on the left sinus. Bone biopsies were harvested from the sinus during implant site preparation with the aid of a trephine drill (3i Implant Innovations, Florida, FL, USA) (3.0 mm in diameter and 10 mm in length). After implant placement, the implant stability quotient (ISQ) was measured using a RFA device (Osstell; Integration Diagnostics, Gothenburg, Sweden) in the mesiodistal and buccal-palatal regions and the mean values were used, as described previously.[22, 24] For the left sinus, two dental implants ((TitamaxTi EX Acqua 4 x 11 mm; internal exagon, Neodent, Curitiba, PR, Brazil) were installed after 8 months post-surgical augmentation procedure (4 months after implant installation in the right sinus). Bone biopsies were harvested and RFA analysis was performed, as described above.

Two months after the last surgical technique (10 months after sinus floor augmentation), prosthetic procedures were carried out to fabricate the definitive prosthesis. At this time, implant stability were measured using the RFA apparatus and the data were recorded. Augmented bone height (4 and 8 months after sinus lifting procedure) was evaluated using volumetric slices achieved with the CBCT scan (SCANORA® 3Dx, Soredex, Tuusula, Finland). The parameters used were as follows: 10 mAs, 90 kVp and a 20 sec scan time using the 9 inch field of view (FOV). The images were exported in DICOM format and reconstructed using specific software (Planmeca, USA). Then, the analyses were evaluated based on the volumetric dimensions automatically calculated by the software.

Biopsies were immediately fixed in 10% buffered formaldehyde solution for 3 days and then processed, as described elsewhere.[23] Serial sections of 6-µm thickness were obtained using an automatic microtome (Jung Supercut 2065, Leica Instruments GmbH, Heidelberg, Germany), mounted on slides and stained with hematoxylin and eosin (H/E). The histological evaluation was made using an optical microscope (Diastar; Leica microsystems GmbH, Wetzlar, Germany) at 100× magnifications. Images were selected and transferred to a

computer display through a digital camera attached to the optical microscope (DFC-300-FX, Leica microsystems GmbH, Wetzlar, Germany) allowing histomorphometric analysis in a specific software (Image J 1.45; Wayne Rasband National Institutes of Health, USA). Histomorphometric analysis was performed to measure the newly formed bone, the reminiscent bone graft, and the amount of fibrous tissue after sinus floor augmentation. Selected slides for histomorphometric analysis followed the semi-series standard: the first section of the first slide was selected, and then four sections sequencing were despised and so on.

CLINICAL OUTCOMES

During the healing period, the patient did not wear any provisional removable denture. No complications were observed during or after the sinus augmentation procedure. No perforation of the sinus membrane was verified, and none of the implants inserted was lost during the follow-up period. The final prosthesis was delivered 10 months after the sinus augmentation procedure (Fig. 5A). CBCT evaluation showed an increased bone resorption in the sinus filled with L-PRF and DBBM compared to the left sinus (22.52% and 8.95% respectively) (Table 1). ISQ were higher than 68 for all implants tested in all the time points (Table 2). Histomorphometric analysis (Fig. 6A-D and table 3) showed higher proportion of newly formed bone in the sinus filled with L-PRF compared to the contralateral side (2118102 and 975535 mm^3). Moreover, the addition of L-PRF allowed fast healing process evidenced by the higher amount of neoformed bone and less fibrous tissue in the sinus, compared to the group without L-PRF (Fig. 6A-D). Osseointegration of dental implants installed after 4 months in the right sinus was successfully achieved. Six months after functional loading, stable bone levels were accomplished with the employed protocols.

DISCUSSION

The findings of this case report demonstrated that the addition of L-PRF to the DBBM graft on the maxillary sinus accelerated bone healing allowing early placement of dental implants (4 months after sinus augmentation compared to the conventional period of 8 months when DBBM is used alone). Moreover, histomorphometric analysis showed higher amount of newly formed bone (Fig. 6C-D) when the growth factor was applied (Table 3). RFA analysis confirmed high ISQ values for the implants installed in both sinus cavities, which represents an adequate implant stability,[22, 24] immediately after implant placement and before prosthesis installation (Table 2). However, a decrease in bone volume was evidenced by CBCT when L-PRF was added into the sinus (Table 1).

The increased bone resorption in the sinus filled with L-PRF might be explained by: compression force during the insertion of the graft in the sinus cavity, surgical technique, and the height of residual bone. Furthermore, lower amount of DBBM was applied to the sinus when associated with the L-PRF. Paralleling clinical data from previous study,[25] the resorption rate when DBBM is used vary between 13,9 and 26% after 6 months post-surgery, which close resemble our findings. According to a previous study,[7] there is no significant relationship between the resorption of grafted bone and the implant success rate (osseointegration) due to the increased stability of the graft over time, especially after 12 months post-operative. Indeed, the bone graft in the sinus filled with L-PRF was replaced earlier by newly formed bone as demonstrated by the blue color in the representative image of the bone biopsy (Fig. 6D). Interestingly, the lower amount of graft material used to fill the sinus floor is an important consideration that should be take into account when L-PRF is used concomitantly with the graft material. From a practical standpoint, the L-PRF is easy to use on the maxillary sinus, and the elastic consistency of the L-PRF membrane allows the clinician to easily insert it inside the sinus floor together with the graft material.

The beneficial effects of L-PRF on tissue healing by means of release of growth factors, and enhancement of angiogenesis and osteogenesis might explain the higher amount

of newly formed bone in the sinus filled with the combination of bone graft and L-PRF, which are in agreement with a recent systematic review.[4] Accordingly, Zhang et al.[26] compared the association with L-PRF and bovine bone graft with only bovine bone in sinus augmentation. The results showed that the percentage of newly formed bone in the PRF group was higher compared to the control group (18.35 ± 5.62% vs 12.95 ± 5.33%). Also, the percentage of residual graft in the PRF group was about 1.5-fold lower than in the control group (28.54 ± 12.01% vs 19.16 ± 6.89%), which close resemble our findings. On the other hand, recent studies[8, 26] have showed that the addition of L-PRF to the DBBM on bone augmentation in maxillary sinus did not increase the percentage of newly formed bone after 6 months post-surgery. Recent study[27] evaluated the efficacy of using L-PRF combined with anorganic bovine bone graft (ABBG) (test group) in a 2-stage maxillary sinus augmentation procedure compared to the ABBG alone (control group). The authors showed that there were no differences related to new bone formation when L-PRF was added (35.0 ± 8.6 compared to 32.97 ± 9.71 of control group). Additionally, no differences were noted in regard to the amount of connective tissue and biomaterial reminiscent (33.05 ± 6.29 in the test group; 33.79 ± 8.57 in the control group). These findings could be attributed to the differences in the study design, patient characteristics, surgical protocol, healing period, and sample size.

The findings of the present case report suggested that implant placement might be installed earlier than the standard time of 8 months in grafted areas in the posterior region of the maxilla. Evidently, further longitudinal, randomized, controlled clinical trials are warranted to support this assumption. Similar to this clinical outcomes, Choukroun et al[28] evaluated the success of PRF and freeze-dried bone allograft (FDBA) mixture for sinus floor augmentation. The authors demonstrated that the combination of L-PRF and FDBA reduced the healing time prior to implant installation. They claimed that the healing time could be reduced to 4 months by using PRF, which parallel our findings. According to the authors,[28] PRF does not seem to improve cellular proliferation, but may play a crucial role in the graft

revascularization by supporting angiogenesis, and acting as a bio-barrier protecting the graft material.[29] Furthermore, by release of growth factors and leukocytes, the L-PRF stimulates neoangiogenesis and accelerates tissue healing.

To evaluate the progression of implant stability, RFA was employed immediately after implant placement and before prosthesis installation throughout measurement of the ISQ as a function of firmness of the bone-to-implant interface. Recent observations[22, 24] have demonstrated that average values of ISQ around 67 represent great implant stability and a complete process of osseointegration. Despite the treatment employed is this case, all implants presented with high ISQ values demonstrating great implant stability allowing the prosthesis installation. A recent study[30] evaluated the effect of L-PRF on bone healing around dental implants installed in the posterior area of the maxilla. ISQ was assessed by RFA after 2, 4 and 6 weeks after implant placement. They showed increased ISQ values when L-PRF was added during surgery for all time points evaluated. The authors suggest that the addition of L-PRF might enhance the post-insertion stability of implants during implant healing. Taken together, the findings presented in this case might suggest the efficacy of L-PRF in accelerate bone healing allowing early placement of dental implants.

CONCLUSION

This report demonstrates the advantages of adding L-PRF to the DBBM for maxillary sinus augmentation. The increased new bone formation between these 2 groups (DBBM alone and DBBM + L-PRF) make it possible to consider sinus floor augmentation with a shorter healing period before implant installation (4 months instead of 8 months). Furthermore, the quantity of bone material used to fill the sinus cavity can be safely reduced without compromising the final graft volume. Nevertheless, further longitudinal, prospective and controlled clinical trials should be performed before justifying the predictability and generalizability for the use of L-

PRF and to validate the healing time of 4 months between sinus augmentation and implant installation.

List of abbreviations

L-PRF: Leukocyte and Platelet Rich Fibrin

DBBM: Demineralized Bovine Bone Mineral

FDBA: Freeze-Dried Bone Allograft

ABBG: Anorganic Bovine Bone Graft

CM: Collagen Membrane

CBCT: Cone Beam Computed Tomography

RFA: Resonance Frequency Analysis

ISQ: Implant Stability Quotient

DICOM: Digital Imaging and Communications in Medicine

H&E: Hematoxylin and Eosin

Conflict of Interest:

The authors report no conflicts of interest related to this case report.

REFERENCES:

1. Boyne PJ, James RA. Grafting of the maxillary sinus floor with autogenous marrow and bone. *J Oral Surg* 1980;38:613-616.
2. Browaeys H, Bouvry P, De Bruyn H. A literature review on biomaterials in sinus augmentation procedures. *Clin Implant Dent Relat Res* 2007;9:166-177.
3. Tadjoedin ES, de Lange GL, Bronckers AL, Lyaruu DM, Burger EH. Deproteinized cancellous bovine bone (Bio-Oss) as bone substitute for sinus floor elevation. A

retrospective, histomorphometrical study of five cases. *J Clin Periodontol* 2003;30:261-270.

4. Castro AB, Meschi N, Temmerman A, et al. Regenerative potential of leucocyte- and platelet-rich fibrin. Part B: sinus floor elevation, alveolar ridge preservation and implant therapy. A systematic review. *J Clin Periodontol* 2017;44:225-234.
5. De Pascale MR, Sommese L, Casamassimi A, Napoli C. Platelet derivatives in regenerative medicine: an update. *Transfus Med Rev* 2015;29:52-61.
6. Eren G, Atilla G. Platelet-rich fibrin in the treatment of localized gingival recessions: a split-mouth randomized clinical trial. *Clin Oral Investig* 2014;18:1941-1948.
7. Kim ES, Moon SY, Kim SG, Park HC, Oh JS. Three-dimensional volumetric analysis after sinus grafts. *Implant Dent* 2013;22:170-174.
8. Nizam N, Eren G, Akcali A, Donos N. Maxillary sinus augmentation with leukocyte and platelet-rich fibrin and deproteinized bovine bone mineral: A split-mouth histological and histomorphometric study. *Clin Oral Implants Res* 2017.
9. Ocak H, Kutuk N, Demetoglu U, Balcioglu E, Ozdamar S, Alkan A. Comparison of Bovine Bone-Autogenic Bone Mixture Versus Platelet-Rich Fibrin for Maxillary Sinus Grafting: Histologic and Histomorphologic Study. *J Oral Implantol* 2017;43:194-201.
10. Chang IC, Tsai CH, Chang YC. Platelet-rich fibrin modulates the expression of extracellular signal-regulated protein kinase and osteoprotegerin in human osteoblasts. *J Biomed Mater Res A* 2010;95:327-332.
11. Oncu E, Kaymaz E. Assessment of the effectiveness of platelet rich fibrin in the treatment of Schneiderian membrane perforation. *Clin Implant Dent Relat Res* 2017;19:1009-1014.

12. Kotsakis GA, Boufidou F, Hinrichs JE, Prasad HS, Rohrer M, Tosios KI. Extraction Socket Management Utilizing Platelet Rich Fibrin: A Proof-of-Principle Study of the "Accelerated-Early Implant Placement" Concept. *J Oral Implantol* 2016;42:164-168.

13. Ali S, Bakry SA, Abd-Elhakam H. Platelet-Rich Fibrin in Maxillary Sinus Augmentation: A Systematic Review. *J Oral Implantol* 2015;41:746-753.

14. Rutkowski JL. Evolution of bone grafting for improved predictability. *J Oral Implantol* 2014;40:1-2.

15. Dos Santos PL, de Molon RS, Queiroz TP, et al. Evaluation of bone substitutes for treatment of peri-implant bone defects: biomechanical, histological, and immunohistochemical analyses in the rabbit tibia. *J Periodontal Implant Sci* 2016;46:176-196.

16. de Molon RS, de Avila ED, Cirelli JA, et al. A Combined Approach for the Treatment of Resorbed Fresh Sockets Allowing Immediate Implant Restoration: A 2-Year Follow-Up. *J Oral Implantol* 2015;41:712-718.

17. de Molon RS, Sakakura CE, Faeda RS, et al. Effect of the long-term administration of Cyclosporine A on bone healing around osseointegrated titanium implants: A histomorphometric study in the rabbit tibia. *Microsc Res Tech* 2017;80:1000-1008.

18. de Molon RS, Morais-Camilo JA, Verzola MH, Faeda RS, Pepato MT, Marcantonio E, Jr. Impact of diabetes mellitus and metabolic control on bone healing around osseointegrated implants: removal torque and histomorphometric analysis in rats. *Clin Oral Implants Res* 2013;24:831-837.

19. Davis VL, Abukabda AB, Radio NM, et al. Platelet-rich preparations to improve healing. Part I: workable options for every size practice. *J Oral Implantol* 2014;40:500-510.

20. Davis VL, Abukabda AB, Radio NM, et al. Platelet-rich preparations to improve healing. Part II: platelet activation and enrichment, leukocyte inclusion, and other selection criteria. *J Oral Implantol* 2014;40:511-521.

21. Ghanaati S, Booms P, Orlowska A, et al. Advanced platelet-rich fibrin: a new concept for cell-based tissue engineering by means of inflammatory cells. *J Oral Implantol* 2014;40:679-689.

22. Dos Anjos TL, de Molon RS, Paim PR, Marcantonio E, Marcantonio E, Jr., Faeda RS. Implant stability after sinus floor augmentation with deproteinized bovine bone mineral particles of different sizes: a prospective, randomized and controlled split-mouth clinical trial. *Int J Oral Maxillofac Surg* 2016;45:1556-1563.

23. de Molon RS, de Paula WN, Spin-Neto R, et al. Correlation of fractal dimension with histomorphometry in maxillary sinus lifting using autogenous bone graft. *Braz Dent J* 2015;26:11-18.

24. de Molon RS, Lages FS, Rivera CP, de Souza Faloni AP, Margonar R, Queiroz TP. Evaluation of Short and Regular Implants after Prosthesis Placement in the Mandible: A Nonrandomized Controlled Clinical Trial. *J Contemp Dent Pract* 2017;18:1122-1129.

25. Kirmeier R, Payer M, Wehrschuetz M, Jakse N, Platzer S, Lorenzoni M. Evaluation of three-dimensional changes after sinus floor augmentation with different grafting materials. *Clin Oral Implants Res* 2008;19:366-372.

26. Zhang Y, Tangl S, Huber CD, Lin Y, Qiu L, Rausch-Fan X. Effects of Choukroun's platelet-rich fibrin on bone regeneration in combination with deproteinized bovine bone mineral in maxillary sinus augmentation: a histological and histomorphometric study. *J Craniomaxillofac Surg* 2012;40:321-328.

27. Bolukbasi N, Ersanli S, Keklikoglu N, Basegmez C, Ozdemir T. Sinus Augmentation With Platelet-Rich Fibrin in Combination With Bovine Bone Graft Versus Bovine

Bone Graft in Combination With Collagen Membrane. *J Oral Implantol* 2015;41:586-595.

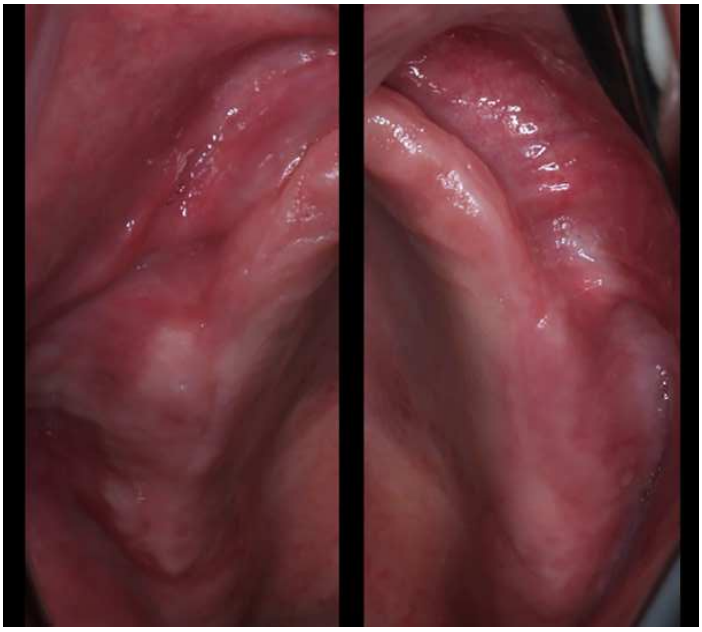
28. Choukroun J, Diss A, Simonpieri A, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part V: histologic evaluations of PRF effects on bone allograft maturation in sinus lift. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006;101:299-303.

29. Del Corso M, Mazor Z, Rutkowski JL, Dohan Ehrenfest DM. The use of leukocyte- and platelet-rich fibrin during immediate postextractive implantation and loading for the esthetic replacement of a fractured maxillary central incisor. *J Oral Implantol* 2012;38:181-187.

30. Tabrizi R, Arabion H, Karagah T. Does platelet-rich fibrin increase the stability of implants in the posterior of the maxilla? A split-mouth randomized clinical trial. *Int J Oral Maxillofac Surg* 2017.

FIGURE LEGENDS:

Figure 1: Pre-operative clinical examination. (A) occlusal; (B) CBCT showing the reminiscent alveolar bone and the sinus anatomy.



(A)

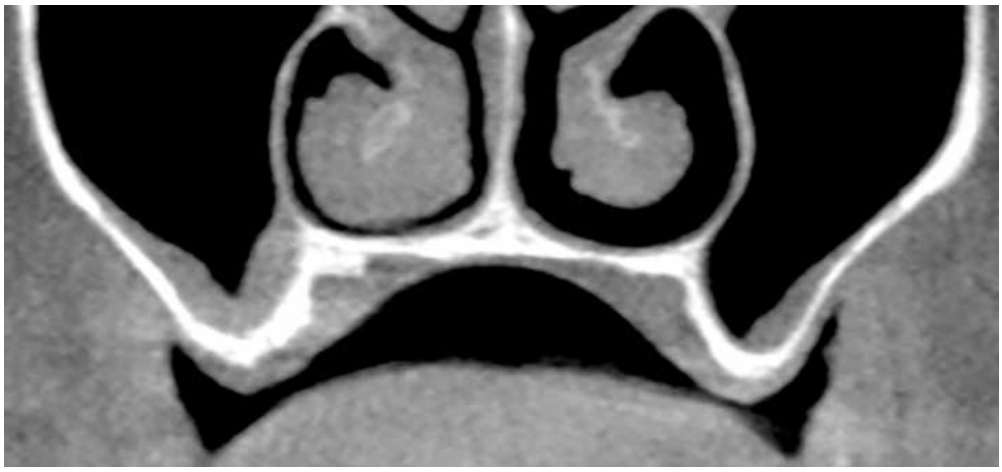


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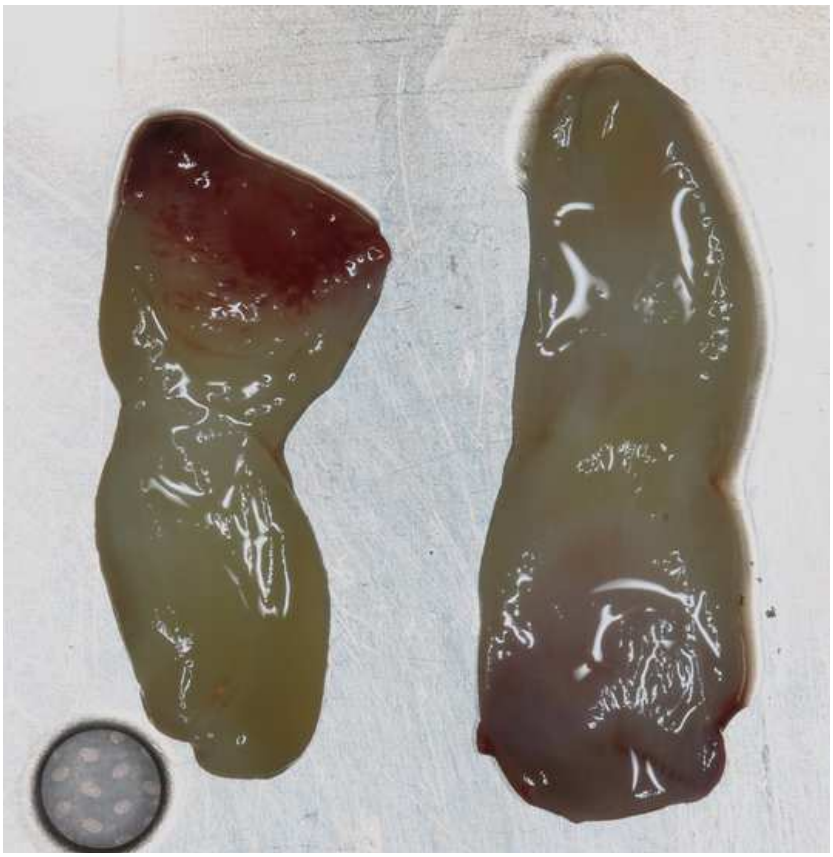
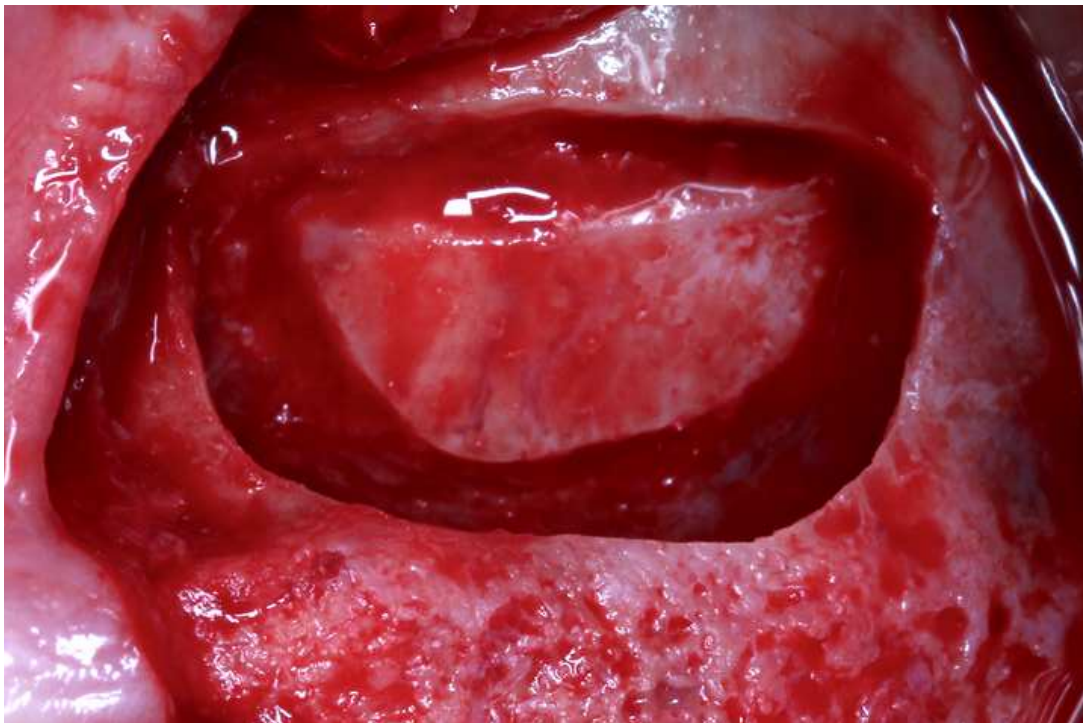
Figure 2: (A) L-PRF clots in the form of a membrane, (B) mixture of L-PRF and DBBM (Bio-Oss®) of 0.25-1 mm particle size.



(A)

(B)

Figure 3: (A) Clinical procedure for sinus floor augmentation using a lateral window approach. (B) Maxillary sinus filled with a combination of L-PRF and Bio-Oss®, and (C) only with DBBM. (D) Resorbable collagen membrane applied over the graft to avoid epithelial cell migration into the maxillary sinus.



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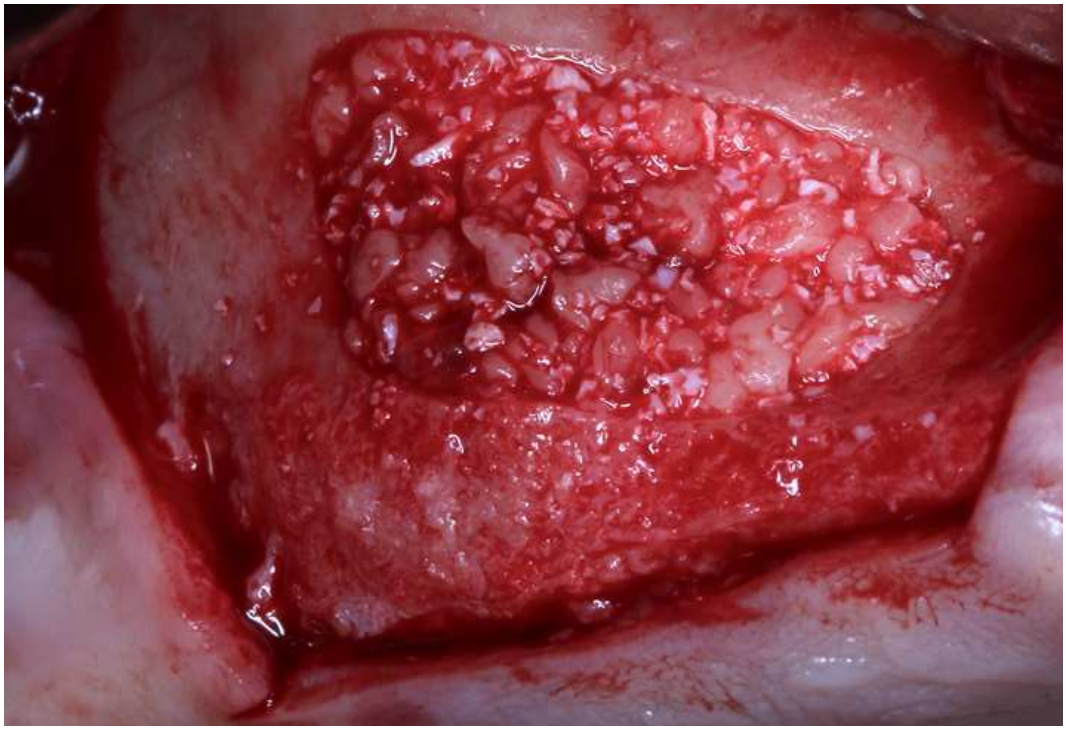

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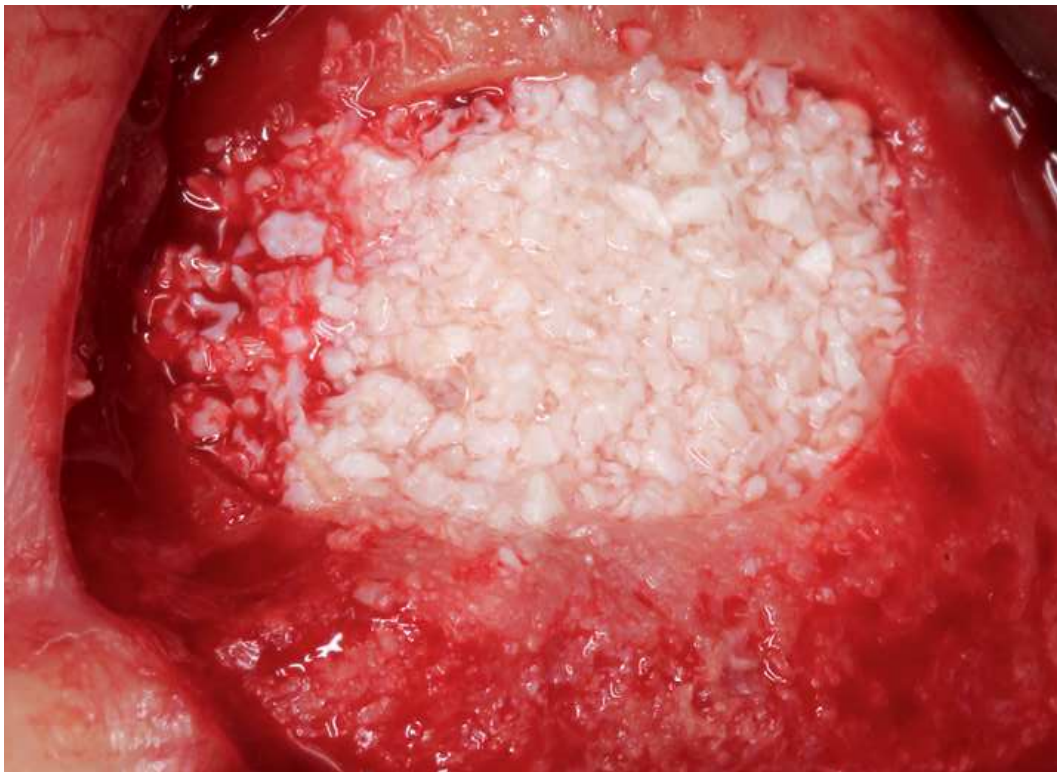

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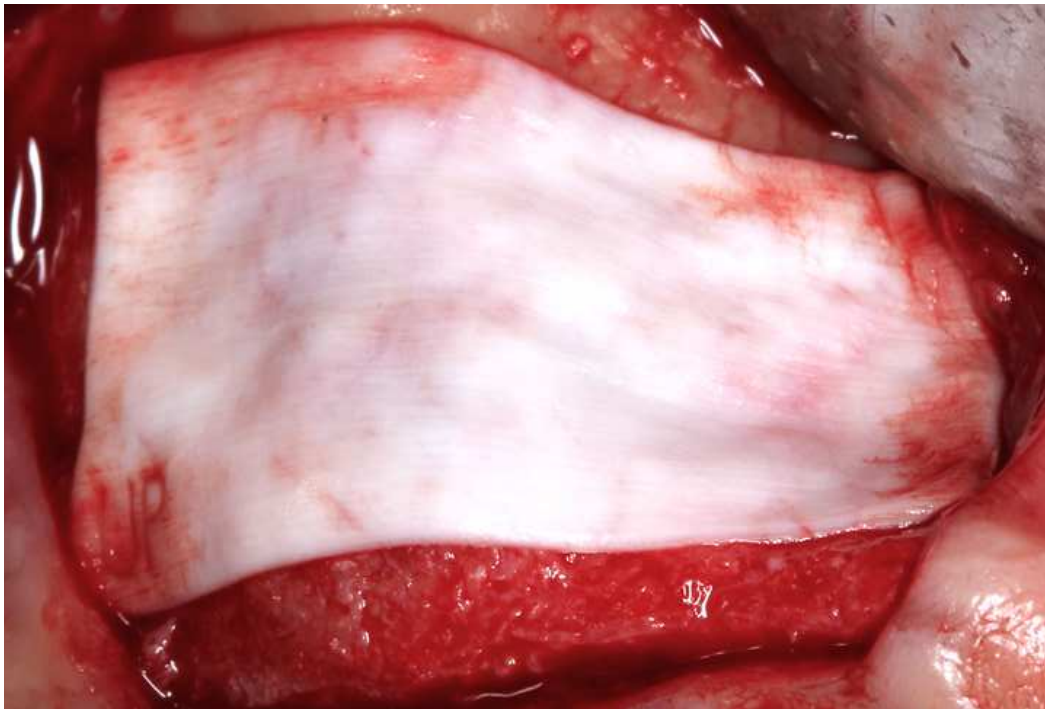
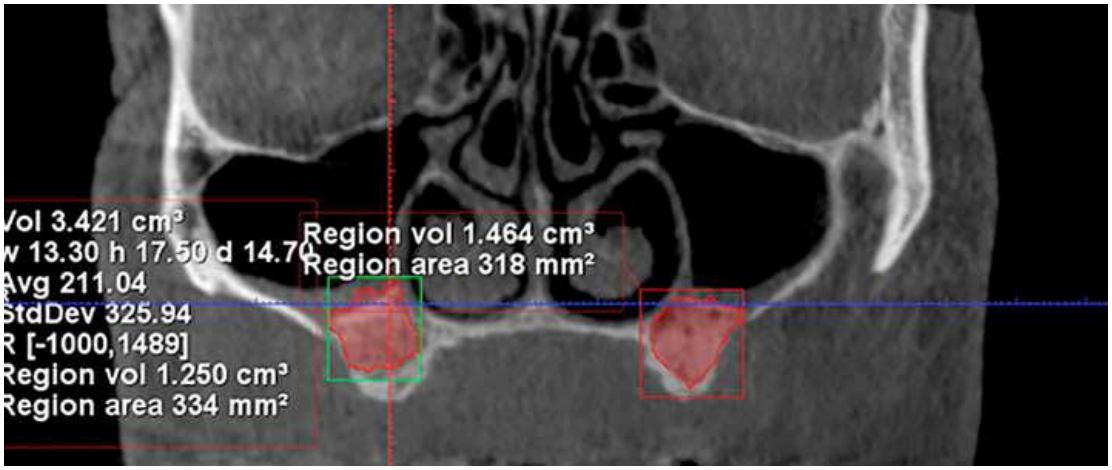


(D)

Figure 4: (A) A new CBCT image acquisition was taken to evaluate the achieved bone volume in the maxillary sinus. (B) Tridimensional reconstruction image achieved from the CBCT.



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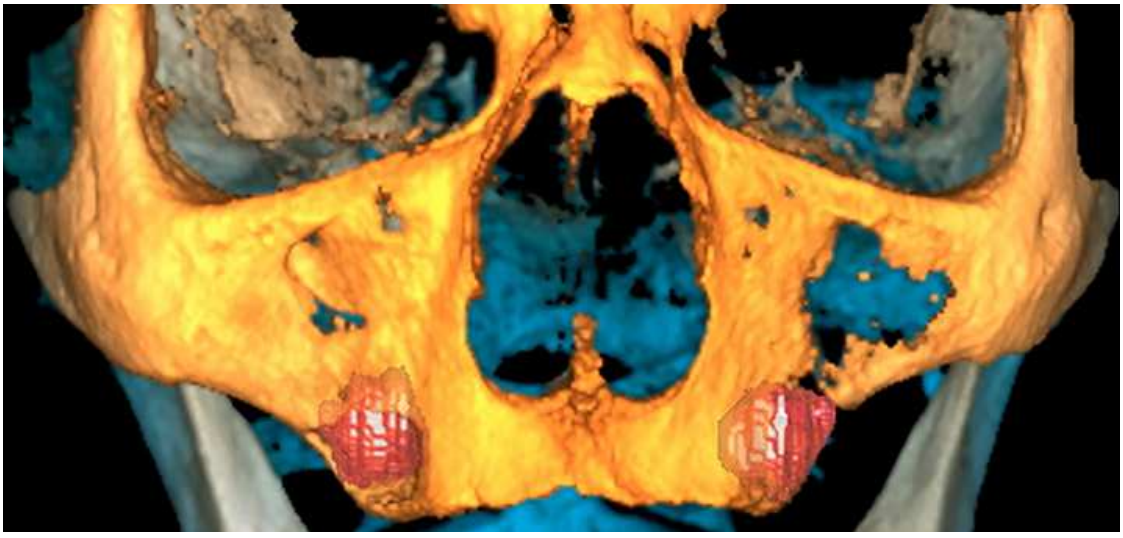


(B)

Figure 5: Final prosthesis delivered after 10 months post sinus augmentation procedure.

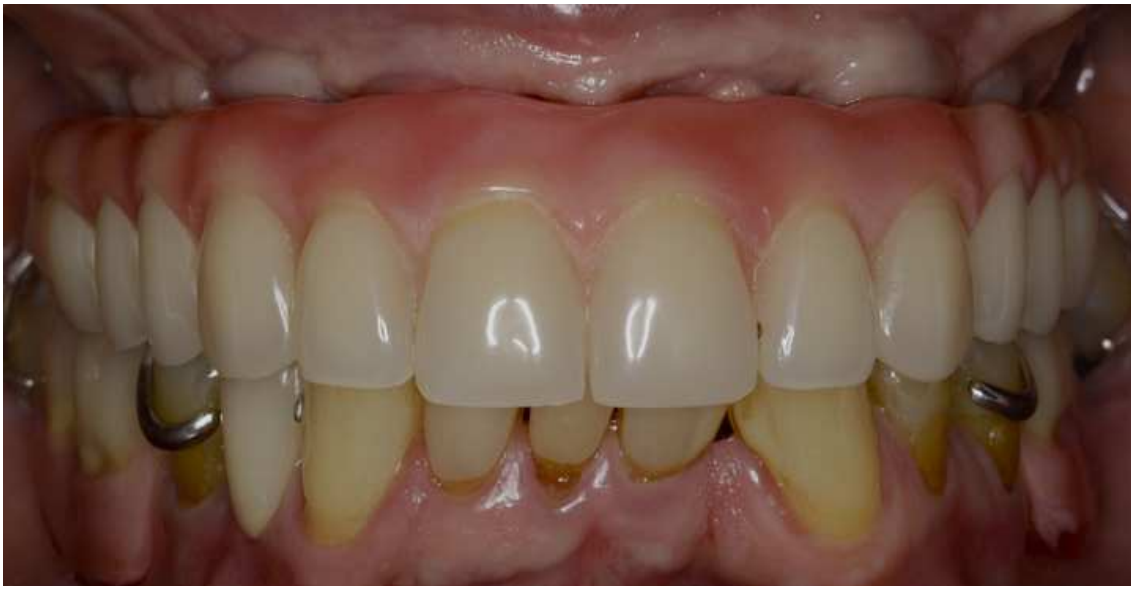
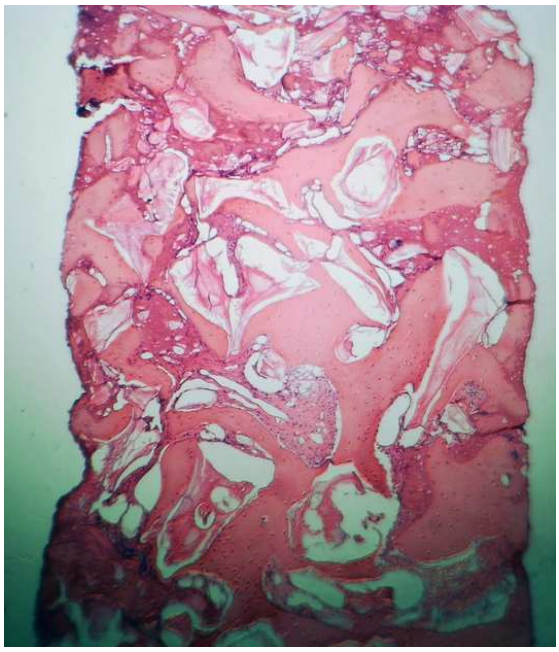


Figure 6: Histomorphometric analysis of the bone biopsies harvested during implant site preparation. (A) Bone biopsy collected from the left maxillary sinus filled with only DBBM. (B) Representative image from the biopsy in which the blue color represents the newly formed bone, green color is fibrous tissue, and orange color is the bone graft material. (C) Bone biopsy collected from the right sinus. (D) Representative image demonstrating increased amount of newly formed bone (blue color) in the sinus filled with L-PRF.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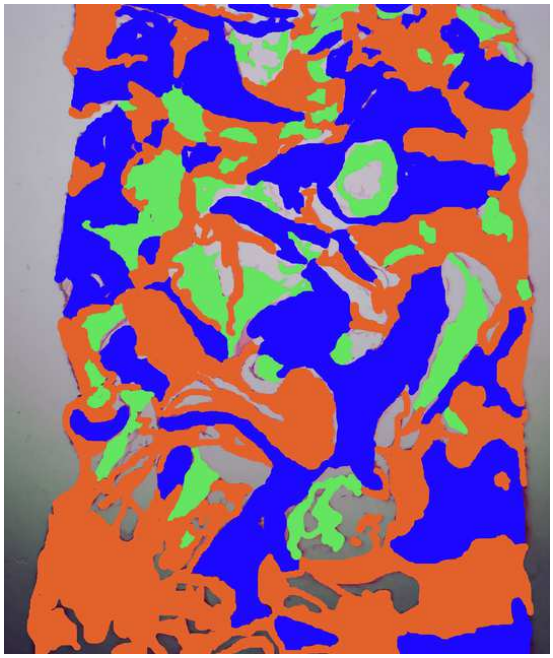
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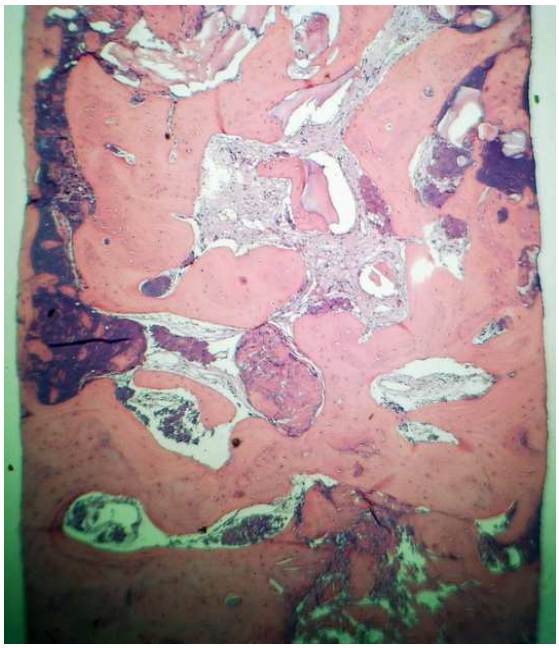
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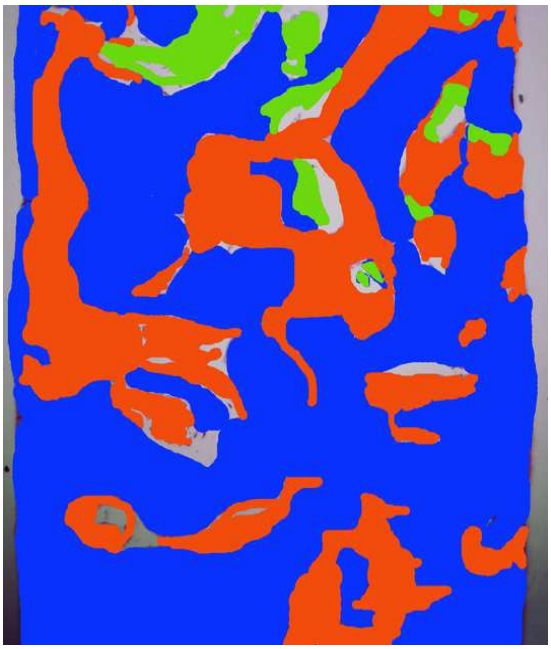

(D)

TABLE LEGENDS

Table 1 – CBCT evaluation after sinus floor augmentation in the right (L-PRF + Bio-Oss) and in the left side (Bio-Oss) of the maxillary sinus. Values were obtained in cm^3 using the Planmeca software.

Volumetric values	Bone volume (cm3)	Resorption
L-PRF + Bio-Oss	Initial: 1.714 4 months: 1.328 Difference: 0.386	22.5%
Bio-Oss	Initial: 1.664 8 months: 1.515 Difference: 0.149	8.95%

Table 2 – ISQ values measured immediately after implant placement (initial) and before prosthesis installation (final) in the buccal-palatal (B-P) and in the medial-distal (M-D) regions of the implants.

Implant	ISQ initial			ISQ final		
	B-P	M-D	Mean	B-P	M-D	Mean
L-PRF + Bio-Oss						
15	70	71	70.5	81	85	83
16	66	71	68.5	80	80	80
Bio-Oss						
25	70	85	77.5	63	85	74
26	70	83	76.5	70	85	77.5

Table 3 – Values of the histological analysis (mm^3) evaluating newly formed bone, the amount of bone graft and the fibrous tissue.

Histologic values	New bone	Bone graft	Fibrous tissue
L-PRF + Bio-Oss	2118102	140151	960575
Bio-Oss	975535	319214	1134752

4 DISCUSSÃO

O potencial da L-PRF em acelerar a regeneração óssea quando usada em combinação com OBD no procedimento de elevação da membrana do seio maxilar tem sido amplamente discutido na literatura. As plaquetas têm efeitos benéficos sobre a cicatrização do tecido duro por fornecer uma série de fatores de crescimento favoráveis à maturação óssea e à qualidade do osso neoformado[45-48]. Nesta tese foram apresentados 3 artigos de estudo clínico randomizado inéditos. Nestes estudos foi utilizado o modelo de boca dividida, em que no grupo teste foi utilizado L-PRF + OBD e no grupo controle, apenas OBD. No primeiro estudo foram avaliadas as alterações volumétricas dos enxertos nos grupos teste e controle após 8 meses de cicatrização. A diferença no percentual de redução do volume ósseo foi significativa entre os grupos; porém não houve uma diferença significativa entre os grupos em relação à estabilidade inicial dos implantes. Como não encontramos na literatura dados sobre a influência da L-PRF na alteração do volume ósseo quando combinada ao OBD por meio de análise tomográfica, subtemos nosso artigo para publicação sem a inclusão da análise histológica. O segundo artigo descreve os resultados da análise histológica das biópsias dos pacientes incluídos no primeiro artigo. A associação de L-PRF + OBD resultou em um aumento de osso recém formado em relação à somente OBD após 8 meses de cicatrização, mas a quantidade e porcentagem de enxerto ósseo residual e tecido fibroso foram semelhantes. Com o terceiro artigo procuramos responder um questionamento bastante relevante para a prática clínica: a L-PRF é capaz de estimular a neoformação e maturação óssea quando combinado ao OBD possibilitando a realização de protocolos precoces de instalação de implantes na região posterior de maxilas atróficas? Dessa forma, reduzimos o tempo de cicatrização após o procedimento de elevação do seio maxilar para 4 meses no grupo enxertado com L-PRF + OBD. Neste artigo apresentamos os resultados de forma completa, com análises clínica, histológica e radiográfica. O grupo testado com 4 meses de cicatrização houve uma maior quantidade de osso recém formado, enquanto o material de enxerto residual foi estatisticamente menor. As mudaças dimensionais volumétricas aconteceram sem diferenças significantes entre os grupos e a estabilidade inicial dos implantes tiveram valores favoráveis à osseointegração. O quarto artigo é um relato de caso clínico completo que descreve desde o

planejamento cirúrgico até o sucesso da reabilitação final do paciente envolvendo tempos diferentes de cicatrização tanto para o enxerto como para os implantes.

Uma grande variedade de protocolos de processamento do sangue para obtenção do biomaterial se desenvolveu após os resultados favoráveis da L-PRF em relação aos tecidos moles e duros[49]. O protocolo preconizado por Choukroun (European Directive no. 2004/23/CE of March 31, 2004) consiste na captação de sangue venoso do paciente antes da cirurgia utilizando tubos de vidro de 10 mL sem adição de anticoagulante e centrifugação imediata em 3000 rpm por 12 minutos. No entanto, outros protocolos podem ser encontrados na literatura como a centrifugação padrão à 2700 rpm por 12 minutos e a centrifugação à 1500 rpm por 14 minutos denominada de PRF Avançado (A-PRF)[50]. Os protocolos de centrifugação visam diferenciar os efeitos da força g gerada pela centrífuga (velocidade e tempo) sobre a distribuição de células relevantes para a cicatrização de feridas e regeneração de tecidos. No nosso estudo, utilizamos o protocolo incialmente proposto por Dohan et al.[24] (2006) por se tratar de uma técnica mais estabelecida na literatura para força e tempo, fatores considerados importantes para obtenção de ótimas membanas[44].

A L-PRF pertence a uma nova geração de concentrados de plaquetas autólogas que apresenta processamento simplificado e sem adição de anticoagulantes. Baseado nos nossos resultados, verificamos que o uso da L-PRF seria uma alternativa viável, segura e financeiramente mais econômica por se tratar de um material derivado do próprio sangue do paciente, que poderia ser utilizada para reduzir o tempo de cicatrização após procedimento de elevação do seio maxilar com OBD.

5 CONCLUSÃO

Dentro dos limites deste estudo, pode-se concluir que:

- A L-PRF acelerou a neoformação óssea quando utilizada em associação com osso bovino desproteinizado no procedimento de elevação do seio maxilar.

- O uso da L-PRF combinada ao bovino desproteinizado resultou em redução significativa do volume ósseo após o período de 8 meses de reparo.

- A estabilidade primária dos implantes não foi afetada de forma significativa pela utilização da L-PRF.

- O uso da L-PRF em combinação com osso bovino desproteinizado pode ser uma alternativa viável para redução do tempo necessário para reabilitação com implantes na região posterior de maxilas atróficas.

REFERÊNCIAS·

1. Cawood JI, Howell RA. A classification of the edentulous jaws. Int J Oral Maxillofac Surg 1988; 17(4): 232–6.

2. Monje A, Monje F, Hernandez-Alfaro F, Gonzalez-Garcia R, Suarez-Lopez del Amo F, Galindo-Moreno P et al. Horizontal bone augmentation using autogenous block grafts and particulate xenograft in the severe atrophic maxillary anterior ridges: a cone-beam computerized tomography case series. J Oral Implantol 2015; 41: 366–71.

3. Schwarz F, Ferrari D, Balic E, Buser D, Becker J, Sager M. Lateral ridge augmentation using equine- and bovine- derived cancellous bone blocks: a feasibility study in dogs. Clin Oral Implants Res 2010; 21(9): 904–12.

4. von Arx T, Buser D. Horizontal ridge augmentation using autogenous block grafts and the guided bone regeneration technique with collagen membranes: a clinical study with 42 patients. Clin Oral Implants Res 2006; 17(4): 359–66.

5. Renouard F, Nisand D. Short implants in the severely resorbed maxilla: a 2-year retrospective study. Clin Implant Dent Relat Res. 2005; 7(Suppl 1): S104-S110.

6. Ridell A, Gröndahl K, Sennerby L. Placement of Brånemark implants in the maxillary tube region: anatomical considerations, surgical technique and long term results. Clin Oral Implants Res. 2009; 20: 94-8.

7. Breine U, Brånemark PI. Reconstruction of alveolar jaw bone. Scand J Plast Reconstr Surg. 1980;14: 14-23.

8. Boyne PJ, Lames RA. Grafting of the maxillary sinus floor with autogenous marrow and bone. J Oral Surg. 1980; (8)38: 613-6.

9. Tatum H. Maxillary and sinus implant reconstruction. Dent Clin North Am. 1986; 30(6): 207-29.

10. Albrektsson T, Johansson C. Osteoinduction, osteoconduction and osseointegration. Eur Spine J. 2001; 10(Suppl 2): S96-S101.

11. Almasri M, Altalibi M. Efficacy of reconstruction of alveolar bone using analloplastic hydroxyapatite tricalcium phosphate graft under biodegradable chambers. Br J Oral Maxillofac Surg. 2011; 49(6): 469-73.

12. Frenken JW, Bouwman WF, Bravenboer N, Zijderveld SA, Schulten EA, ten Bruggenkate CM. The use of Straumann Bone Ceramic in a maxillary sinus floor elevation procedure: a clinical, radiological, histological and histomorphometric evaluation with a 6-month healing period. Clin Oral Implants Res. 2010; 21(2): 201-8.

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

13. Kolerman R, Tal H, Moses O. Histomorphometric analysis of newly formed bone after maxillary sinus floor augmentation using ground cortical bone allograft and internal collagen membrane. J Periodontol. 2008; 79(11): 2104-11.

14. Hallman M, Lundgren S, Sennerby L. Histologic analysis of clinical biopsies taken 6 months and 3 years after maxillary sinus floor augmentation with 80% bovine hydroxyapatite and 20% autogenous bone mixed with fibrin glue. Clin Implant Dent Relat Res. 2001; 3(2): 87-96.

15. Jodia K, Sadhwani BS, Parmar BS, Anchlia S, Sadhwani SB. Sinus elevation with an alloplastic material and simultaneous implant placement: a 1-stage procedure in severely atrophic maxillae. J Maxillofac Oral Surg. 2014; 13(3): 271-80.

16. Al-Nawas B, Schiegnitz E. Augmentation procedures using bone substitute materials or autogenous bone - a systematic review and meta-analysis. Eur J Oral Implantol. 2014; 7(2): 219-34.

17. Clark RA. Fibrin and wound healing. Ann. N.Y. Acad Sci. 2001; 936: 355-67.

18. Bhanot S, Alex JC. Current applications of platelet gels in facial plastic surgery. Facial Plast Surg 2002; 18(1): 27–33.

19. Jakse N, Tangl S, Gilli R, Berghold A, Lorenzoni M, Eskici A et al. Influence of PRP on autogenous sinus grafts. An experimental study on sheep. Clin Oral Implants Res. 2003; 14(5): 578-83.

20. Schaaf H, Streckbein P, Lendeckel S, Heidinger K, Görtz B, Bein G, Boedeker RH et al. Topical use of platelet-rich plasma to influence bone volume in maxillary augmentation: a prospective randomized trial. Vox Sang. 2008; 94(1): 64-9.

21. Schaaf H, Streckbein P, Lendeckel S, Heidinger KS, Rehmann P, Boedeker RH, et al. Sinus lift augmentation using autogenous bone grafts and platelet-rich plasma: radiographic results. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2008; 106(5): 673-8.

22. Schlegel KA, Zimmermann R, Thorwarth M, Neukam FW, Klongnoi B, Nkenke E et al. Sinus floor elevation using autogenous bone or bone substitute combined with platelet-rich plasma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007; 104(3): e15-25.

23. Dohan Ehrenfest DM, de Peppo GM, Doglioli P, Sammartino G. Slow release of growth factors and thrombospondin-1 in Choukroun's platelet-rich fibrin (PRF): a gold standard to achieve for all surgical platelet concentrates technologies. Growth Factors. 2009; 27(1): 63-9.

24. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J et al. Platelet- rich fibrin (PRF): a second –generation platelet concentrate. Part I: technological concepts and evolution. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006; 101(3): e37-44.

25. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part II: platelet-related biologic features. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006; 101(3): e45-50.

26. Gibble JW, Ness PM. Fibrin glue: the perfect operative sealant? Transfusion 1990; 30(8): 741-7.

27. Choukroun J, Adda F, Schoeffer C, Vervelle A. Une opportunite en paro-implantologie: le PRF. Implantodontie. 2001; 42: 55-62.

28. Choukroun J, Diss A, Simonpieri A, Girard M, Schoeffler C, Dohan SL et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part IV: clinical effects on tissue healing. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006; 101(3): e56-60.

29. Choukroun J, Diss A, Simonpieri A, Girard M, Schoeffler C, Dohan SL, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part V: histologic evaluations of PRF effects on bone allograft maturation in sinus lift. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006;101(3):299-303.

30. Dohan Ehrenfest DM, Rasmusson L, Albrektsson T. Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). Trends Biotechnol 2009;27(3):58-67.

31. Anitua E. Plasma rich in growth factors: preliminary results of use in the preparation of future sites for implants. Int J Oral Maxillofac Implants. 1999;14(4):529-35.

32. Kumar RV, Shubhashini N. Platelet rich fibrin: a new paradigm in periodontal regeneration. Cell Tissue Bank. 2013;14(3):453-63.

33. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part II: platelet-related biologic features. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006;101(3):E45-50.

34. Bielecki T, Cieslik-Bielecka A, Zelawski M, Mikusek W. A side-effect induced by the combination of a demineralized freeze-dried bone allograft and leucocyte and platelet-rich plasma during treatment for large bone cysts: a 4-year follow-up clinical study. Transfus Apher Sci. 2012;47(2):133-8.

35. Tatullo M, Marrelli M, Cassetta M, Pacifici A, Stefanelli LV, Scacco S, et al. Platelet rich fibrin (P.R.F.) in reconstructive surgery of atrophied maxillary bones: clinical and histological evaluations. Int J Med Sci. 2012;9(10):872-80.

36. Kim ES, Park EJ, Choung PH. Platelet concentration and its effect on bone formation in calvarial defects: an experimental study in rabbits. J Prosthet Dent. 2001;86(4):428-33.

37. Mazor Z, Horowitz RA, Del Corso M, Prasad HS, Rohrer MD, Dohan Ehrenfest DM. Sinus floor augmentation with simultaneous implant placement using Choukroun's platelet-rich fibrin as the sole grafting material: a radiologic and histologic study at 6 months. J Periodontol. 2009; 80(12):2056-64.

38. Simonpieri A, Choukroun J, Del Corso M, Sammartino G, Dohan Ehrenfest DM Simultaneous sinus-lift and implantation using microthreaded implants and leukocyte- and platelet rich fibrin as sole grafting material: a six year experience. Implant Dent. 2011;20(2):2-12.

39. Kim BJ, Kwon TK, Baek HS, Hwang DS, Kim CH, Chung IK, et al. A comparative study of the effectiveness of sinus bone grafting with recombinant human bone morphogenetic protein 2-coated tricalcium phosphate and platelet-rich fibrin-mixed tricalcium phosphate in rabbits. Oral Surg Oral Med Oral Pathol Oral Radiol. 2012;113(5):583-92.

40. Zhang Y, Tangl S, Huber CD, Lin Y, Qiu L, Rausch-Fan X. Effects of Choukroun's platelet-rich fibrin on bone regeneration in combination with deproteinized bovine bone mineral in maxillary sinus augmentation: a histological and histomorphometric study. J Craniomaxillofac Surg. 2012; 40(4): 321-8.

41. Thorn JJ, Sorensen H, Weis-Fogh U, Andersen M. Autologous fibrin glue with growth factors in reconstructive maxillofacial surgery. Int J Oral Maxillofac Surg. 2004;33(1):95-100.

42. Bölükbaşı N, Yeniyol S, Tekkesin MS, Altunatmaz K. The use of platelet-rich fibrin in combination with biphasic calcium phosphate in the treatment of bone defects: a Histologic and Histomorphometric Study. Curr Ther Res Clin Exp. 2013;75:15-21.

43. Nizam N, Eren G, Akcalı A, Donos N. Maxillary sinus augmentation with leukocyte and platelet-rich fibrin and deproteinized bovine boné mineral: a split-mouth histological and histomorphometric study. Clin Oral Implants Res. 2018;29(1):67-75.

44. Castro AB, Meschi N, Temmerman A, Pinto N, Lambrechts P, Teughels W, et al. Regenerative potential of leucocyte and platelet-rich fibrin. Part B: sinus floor elevation, alveolar ridge preservation and implant therapy. A systematic review. J Clin Periodontol. 2017;44(2):225–234.

45. Chang IC, Tsai CH, Chang YC. Platelet-rich fibrin modulates the expression of extracellular signal-regulated protein kinase and osteoprotegerin in human osteoblasts. J Biomedical Materials Res Part A. 2010;95(1):327–32.

46. De Pascale MR, Sommese L, Casamassimi A, Napoli C. Platelet derivatives in regenerative medicine: an update. Transfus Med Rev. 2015;29(1):52–61.

47. Jeong SM, Lee CU, Son JS, Oh JH, Fang Y, Choi BH. Simultaneous sinus lift and implantation using platelet-rich fibrin as sole grafting material. J Craniomaxillofac Sur. 2014;42(6):990–4.

48. Oliveira MR, de C Silva A, Ferreira S, Avelino CC, Garcia IR Jr, Mariano RC. Influence of the association between platelet- rich fibrin and bovine bone on bone regeneration. A histomorphometric study in the calvaria of rats. Int J Oral Maxillofac Surg. 2015;44(5):649–55.

49. Fujioka-Kobayashi M, Miron RJ, Hernandez M, Kandalam U, Zhang Y, Choukroun J. Optimized platelet-rich fibrin with the low- speed concept: Growth factor release, biocompatibility, and cellular response. J Periodontol. 2017;88(1):112–21.

50. Ghanaati S, Booms P, Orlowska A, Kubesch A, Lorenz J, Rutkowski J, et al. Advanced Platelet-Rich Fibrin (A-PRF) – a new concept for cell-based tissue engineering by means of inflammatory cells. J Oral Implantol. 2014;40(6):679-89.

51. Troedhan A, Schlichting I, Kurrek A, Wainwright M. Primary implant stability in augmented sinuslift-sites after completed bone regeneration: a randomized controlled clinical study comparing four subantrally inserted biomaterials. Sci Rep. 2014;30(4):5877.

52. Harris D, Horner K, Gröndahl K, Jacobs R, Helmrot E, Benic GI, et al. E.A.O. guidelines for the use of diagnostic imaging inimplant dentistry 2011. A consensus workshop organized by the European Association for Osseointegration at the Medical University of Warsaw. Clin Oral Implants Res. 2012;23(11):1243-53.

APÊNDICE A - Materiais e Métodos

O protocolo do presente estudo foi aprovado pelo Comitê de Ética em Pesquisa em Seres Humanos (CEP) da Faculdade de Odontologia de Araraquara – UNESP sob o número CAAE 41357514.5.0000.5416 (ANEXO A).

A.1 População do estudo

Os participantes deste estudo foram recrutados no Curso de Especialização em Implantodontia da Faculdade de Odontologia de Araraquara. Foram selecionados pacientes que buscavam reabilitação oral por meio de implantes osseointegráveis, porém necessitavam de procedimento prévio de enxerto ósseo no seio maxilar para instalação de implantes.

Critérios de inclusão:

* Ter mais de 18 anos
* Edentulismo bilateral na região posterior maxila
* Em relação ao tecido ósseo na região posterior: disponibilidade de largura (mínimo de 5 mm) e altura (até 4mm) verificada por meio de tomografia computadorizada, situação que recomenda a realização de enxerto ósseo do tipo inlay visando a recuperação da altura óssea.
* Assinatura do Termo de Consentimento Livre e Esclarecido do presente estudo

Critérios de Exclusão:

* Tabagismo, Etilismo e Uso de Drogas
* Diabetes não controlada
* Desordens sanguíneas
* Deficiência imunológica
* Uso de bisfosfonatos
* Gravidez
* Utilização de medicamentos que alterem a função plaquetária e que afetem o metabolismo ósseo
*Presença de sinais e sintomas clínicos e de imagens relacionadas à patologias ósseas e sinusais
* Radioterapia prévia na região de cabeça e pescoço
* Pacientes realizando radioterapia ou quimioterapia

Foi utilizado o modelo de boca dividida com o intuito de ter no mesmo paciente os grupos controle e teste. Um total de 24 pacientes foi incluído. Estes pacientes foram submetidos ao procedimento de levantamento do seio maxilar bilateralmente, sendo que no grupo teste o Bio-Oss® foi misturado ao L-PRF e no grupo controle foi utilizado somente o enxerto ósseo de origem bovina. Os participantes do estudo foram randomicamente distribuídos em 2 subgrupos. No subgrupo 1, os implantes foram instalados após 4 meses de cicatrização no grupo teste e após 8 meses no grupo controle. No subgrupo 2, os implantes foram instalados após 8 meses tanto no grupo teste como no controle.

Um único cirurgião experiente realizou todos os procedimentos cirúrgicos. Os materiais de enxerto foram escolhidos aleatoriamente para cada lado em cada participante de acordo com uma lista de randomização gerada por computador. Os números foram selados em envelopes opacos por uma pessoa não envolvida no estudo e sem conhecimento deste ensaio clínico. Assim, o pesquisador que estava realizando a cirurgia somente soube o tratamento que seria realizado no momento exato do procedimento. Uma vez definido o tratamento a ser realizado, a outra forma de tratamento proposta foi realizada no lado contralateral. Os pacientes não foram informados sobre o tipo de tratamento realizado em cada lado da maxila.

A.2 Técnica de obtenção L-PRF

O L-PRF foi preparado como descrito por Dohan et. al.[24] (2006) (European Directive n. 2004/23/CE of March 31th, 2004).

Antes do início da cirurgia, foi realizada a coleta de sangue do paciente. Essa coleta foi realizada por um profissional habilitado para este procedimento, utilizando o sistema de coleta a vácuo - vacutainer (BD Vacutainer® Systems - Divisão Brasil). Foram coletados 4 tubos com capacidade de 10 mL cada sem a adição de anticoagulante ou trombina bovina. Na sequência, os tubos foram centrifugados em 3000 rpm por 10 minutos gerando 300 g de força centrípeda (Kasvi K14-0815, Curitiba, PR, Brasil). Na parte superior do tubo tivemos o plasma acelular (Plasma Pobre em Plaqueta - PPP), na porção inferior as células vermelhas do sangue (hemácias), e na parte intermediária o concentrado de fibrina e componentes celulares do sangue. A ausência de anticoagulante implica na ativação da cascata de coagulação em poucos minutos quando o sangue entra em contato com as paredes do tubo. O fibrinogênio é inicialmente concentrado na parte alta do tubo,

antes da trombina circulante transformá-lo em fibrina. Um coágulo de fibrina é então obtido no meio do tubo, entre os glóbulos vermelhos no fundo e o plasma acelular no topo.

A.3 Técnica cirúrgica de levantamento do seio maxilar

Foi solicitado a todos os pacientes incluídos neste estudo um exame tomográfico volumétrico de feixe cônico da região de interesse para planejamento cirúrgico. Os procedimentos cirúrgicos foram realizados por um único operador experiente. Utilizamos o método descrito por Boyne e James[8] (1980) e Tatum[9] (1986) para elevação da membrana do seio maxilar.

Após a antissepsia extra bucal com PVPI e intra bucal com digluconato de clorexidina a 0,12%, foi realizada a anestesia dos nervos alveolar superior posterior e médio e do nervo palatino maior (cloridrato de articaína com epinefrina 1:100.000, DFL, Brasil), responsáveis respectivamente pela inervação da região posterior da maxila e da mucosa palatina. Foi feita uma incisão crestal sobre a área edêntula do rebordo alveolar e duas incisões relaxantes na região vestibular, mesial e distal à região de interesse. O retalho mucoperiostal foi descolado e rebatido até a exposição completa da parede lateral externa do seio maxilar. No osso vestibular da maxila, uma abertura de acesso à membrana sinusal foi confeccionada com uma fresa esférica nº 2 em baixa rotação, sob irrigação com cloreto de sódio a 0,9%. A mucosa sinusal também foi deslocada das paredes ósseas no interior do seio maxilar, até as dimensões necessárias para o restabelecimento ósseo que permitiria a inserção do material de enxerto. O paciente foi instruído a realizar a manobra de Valsalva a fim de constatar perfurações na membrana. No lado do Grupo Teste foi inserido na cavidade do seio maxilar a membrana de L-PRF associada ao Bio-Oss® (tamanho das partículas 0,25-1 mm), sempre na proporção de 1 membrana de L-PRF para cada 0,5 g de OBD. Na parede vestibular do seio maxilar enxertado foi posicionada a membrana Bio-Gide® (Bio-Gide®, Geistlich Pharma AG, Suiça) para prevenir a migração do material e de células epiteliais. O lado do grupo controle foi enxertado somente com Bio-Oss® e na parede vestibular da cavidade sinusal foi posicionada a membrana de colágeno reabsorvível. Procedimentos de enxertia horizontal não foram realizados. A região foi suturada com fio de nylon 4-0 (Ethicon, Jonhson & Jonhson S.A.) por meio de pontos interrompidos. Os pacientes receberam orientações pós-operatórias e prescrição de medicação sistêmica

constituída por antibiótico (Amoxicilina 500 mg), anti-inflamatório (Nimesulida 100 mg), analgésico (Dipirona 500 mg) e medicação tópica constituída por enxágue com digluconato de clorexidina a 0,12%. A sutura foi removida após 10 dias e a região operada permaneceu sem influência de carga direta durante toda a fase de regeneração óssea. Em todo processo operatório não notamos perfuração visível da membrana sinusal nos casos apresentados. Um novo exame tomográfico foi realizado logo após a cirurgia de enxerto ósseo para determinar o volume ósseo obtido com o procedimento de levantamento da membrana sinusal e enxerto ósseo.

A.4 Instalação de implantes e obtenção da biópsia

Em uma segunda etapa cirúrgica, o leito foi reaberto para instalação de implantes. No subgrupo 1 (n=12), a reabertura foi realizada após 4 meses[31] de cicatrização no grupo teste e após 8 meses[51] no grupo controle. No subgrupo 2 (n=12), a instalação dos implantes foi realizada após 8 meses[51] tanto no grupo teste como no controle. Inicialmente, foi realizada a antissepsia extra e intra-bucal do campo operatório. Após a injeção local de anestésico (Cloridrato de articaína com epinefrina 1:100.000, DFL, Brasil) dos nervos alveolar superior posterior e médio e do nervo palatino maior, foi realizada uma incisão crestal sobre a área edêntula do rebordo alveolar e duas incisões relaxantes na região vestibular, mesial e distal à região de interesse, mesmo procedimento realizado na primeira cirurgia de enxerto ósseo. O retalho mucoperiostal foi descolado e rebatido até a exposição completa da parede lateral externa do seio maxilar. Uma biópsia foi obtida no momento do preparo da loja cirúrgica para instalação dos implantes, por meio de broca trefina (3i Implant Innovations, Florida, EUA) de 2,0 mm de diâmetro interno e 3,0 mm externo. A broca foi posicionada no mesmo eixo de perfuração e inserção dos implantes, justamente nas regiões que os receberiam após o período de neoformação óssea. O corte com a broca foi realizado utilizando contra-ângulo com redução 20:1 (KaVo - KaVo do Brasil S. A. Ind. e Com. - Joinvile - SC) acionada por motor específico (Driller BLM 650 - VK Driller Equipamentos Elétricos Ltda., São Paulo, SP) e refrigeração constante com solução salina, até o rompimento do assoalho do seio maxilar para remoção do osso, na totalidade do seu comprimento. Após a perfuração única, implantes de 4 mm de diâmetro e 11 mm de comprimento modelo TitamaxTi EX ACQUA (Neodent, Curitiba, Brasil) foram inseridos nos leitos preparados e o osso colhido, removido cautelosamente do interior da trefina. As

biópsias foram acondicionadas em solução de formol de Lillie tamponado a 10%.

A.5 Avaliação da estabilidade dos implantes

Após a instalação dos implantes, foi feita a mensuração da estabilidade dos implantes com a utilização do aparelho Osstell® (Osstell AB, Göteborg, Suécia). Este aparelho determina a estabilidade do implante por meio da análise da frequência de ressonância. O sistema inclui a utilização de um SmartPeg™ fixado ao implante através de um parafuso integrado. O SmartPeg™ é excitado por um impulso magnético da sonda de medição do instrumento portátil e o coeficiente de estabilidade do implante (ISQ) é calculado a partir do sinal de resposta. Os resultados são exibidos no instrumento variando numa escala de 1 a 100. Quanto maior o número de ISQ, maior é a estabilidade do implante. As medidas de estabilidade foram obtidas em duas direções, vestibular-palatina e mésio-distal.

A.6 Processamento da biópsia

O processamento foi realizado após a primeira fase de fixação em formol de Lillie (Formol tamponado a 10%) por no mínimo 72 horas para manutenção das estruturas que se deseja avaliar. Na sequência, as peças foram lavadas em água corrente, durante 24 horas, para remoção do formol e foi iniciado o processo de descalcificação das mesmas com sua imersão em solução descalcificadora de Morse (partes iguais de citrato de Sódio a 20% e ácido fórmico a 50%), que foi trocada a cada 48 horas durante 40 dias. Ao constatar a correta descalcificação das peças, as mesmas foram imersas em solução de citrato de sódio a 5% durante três dias consecutivos, com trocas diárias da solução para remoção do excesso de ácido neutralizado e, posteriormente, lavadas em água corrente durante 24 horas antes do início de sua desidratação. A desidratação foi feita em banhos crescentes de álcool (70º, 90º, álcool absoluto), permitindo que a peça seja finalmente diafanizada em xilol durante 3 horas e incluída em parafina. Foram obtidos cortes semi-seriados com aproximadamente 5 µm de espessura ao longo de toda extensão do bloco. A técnica de coloração empregada foi Hematoxilina e Eosina (H/E), permitindo a correta avaliação histológica e histomorfométrica das estruturas teciduais.

A.7 Análise histomorfométrica

Foram utilizadas cinco lâminas em sequência e os cortes selecionados para a

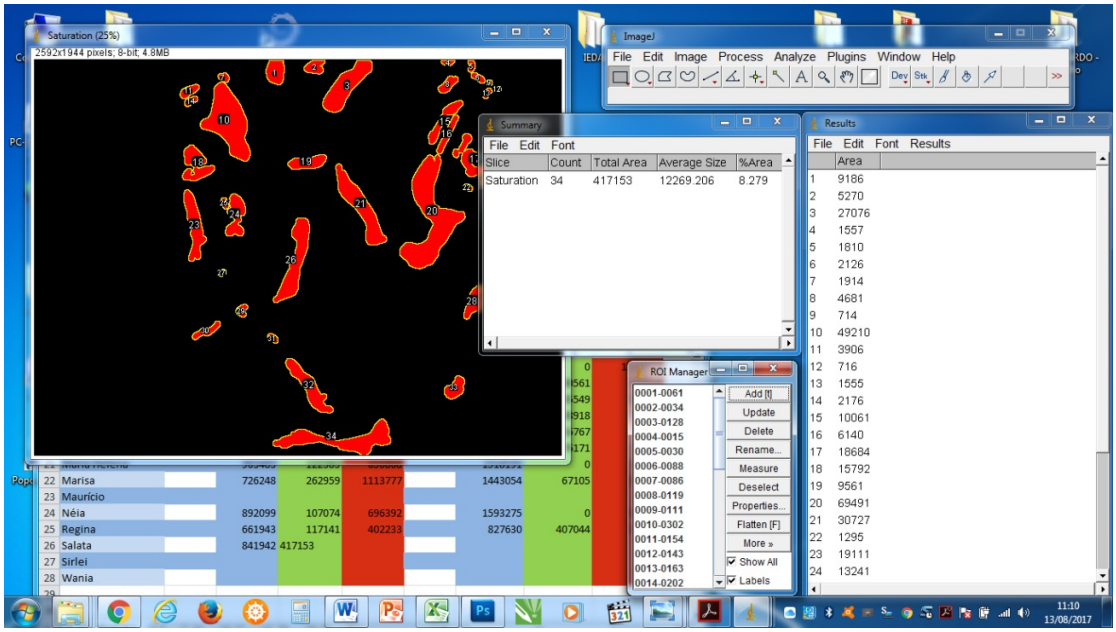
análise histomorfométrica seguiram o seguinte padrão: o primeiro corte da primeira lâmina foi selecionado, e então quatro cortes sequenciais foram desprezados, independentemente de estarem na mesma lâmina, e então o próximo corte foi avaliado, e assim consecutivamente até obter 10 cortes.

Com o auxílio de um microscópio de luz, as lâminas foram posicionadas sobre a porção central dos tecidos (que tinham em média 8 mm de comprimento x 2 mm de largura) contidos nas lâminas a fim de que a imagem capturada não viesse a favorecer a porção óssea nativa do seio maxilar remanescente no processo de coleta (região com grande quantidade de osso vitalizado e nativo do próprio paciente), nem tão pouco a porção mais distante do osso nativo (contendo uma maior probabilidade de menor formação óssea pelo menor estímulo ósseo presente nesta região). Essa padronização foi realizada para todas as laminas analisadas.

As imagens foram capturadas e digitalizadas através de um microscópio óptico (Diastar - Leica eichert & Jung products, Alemanha) em aumento de 5x com uma câmera fotográfica digital (DFC-300-FX, Leica Microsystems, Alemanha) ligada diretamente ao computador por uma porta USB (Figura A2 e A3). As imagens digitalizadas foram submetidas à análise no software Image J 1.45 (Wayne Rasband National Institutes of Health, EUA) para histomorfometria e cálculo da área ocupada por neoformação óssea, tecido fibroso e material de enxerto ósseo bovino remanescente em todos os grupos e períodos (http://rsbweb.nih.gov/ij/download.html) (Figura A1).

Os dados obtidos foram submetidos ao teste de normalidade Teste D'Agostino & Pearson, sendo os dados com distribuição normal foram comparados pelo Teste T (teste pareado). Os dados com distribuição não normal foram analisados pelo Teste de Wilcoxon (teste não paramétrico).

Figura A1 - Histomorfometria - As imagens digitalizadas foram analisadas histologicamente e quantificadas por meio de estudo histomorfométrico com o auxílio de um software gratuito de análise.



Fonte: Software Image J 1.45

Figura A2 - Imagens histológicas obtidas de biópsias das áreas enxertadas de um único paciente bilateralmente (H & E 5x).

A: Imagem histológica do grupo teste do subgrupo 1 (4 meses de cicatrização).

B: Imagem histológica do grupo controle do subgrupo 1 (8 meses de cicatrização).

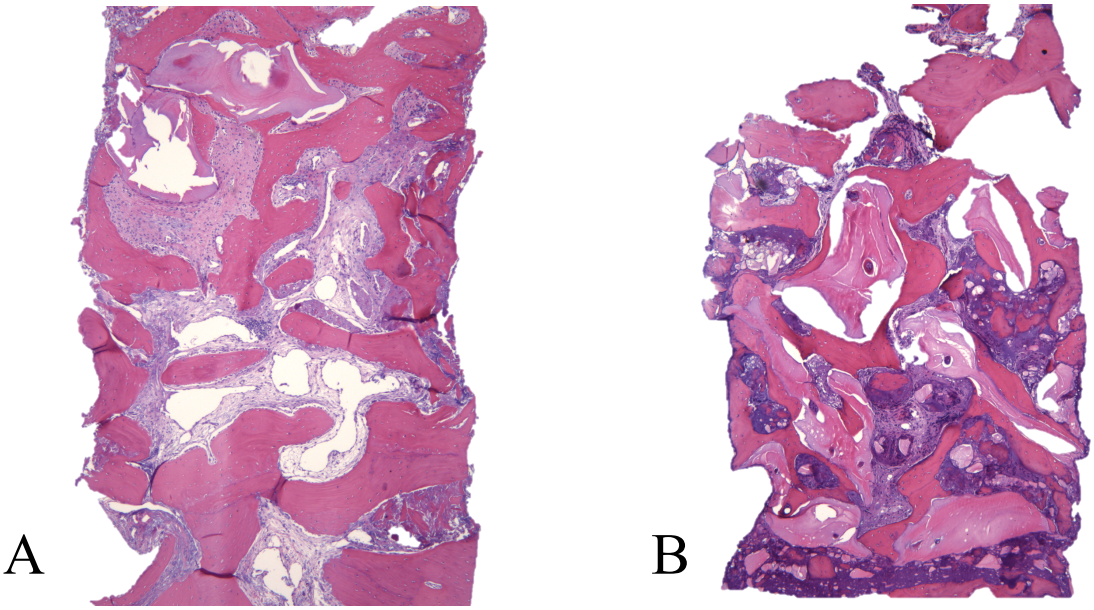
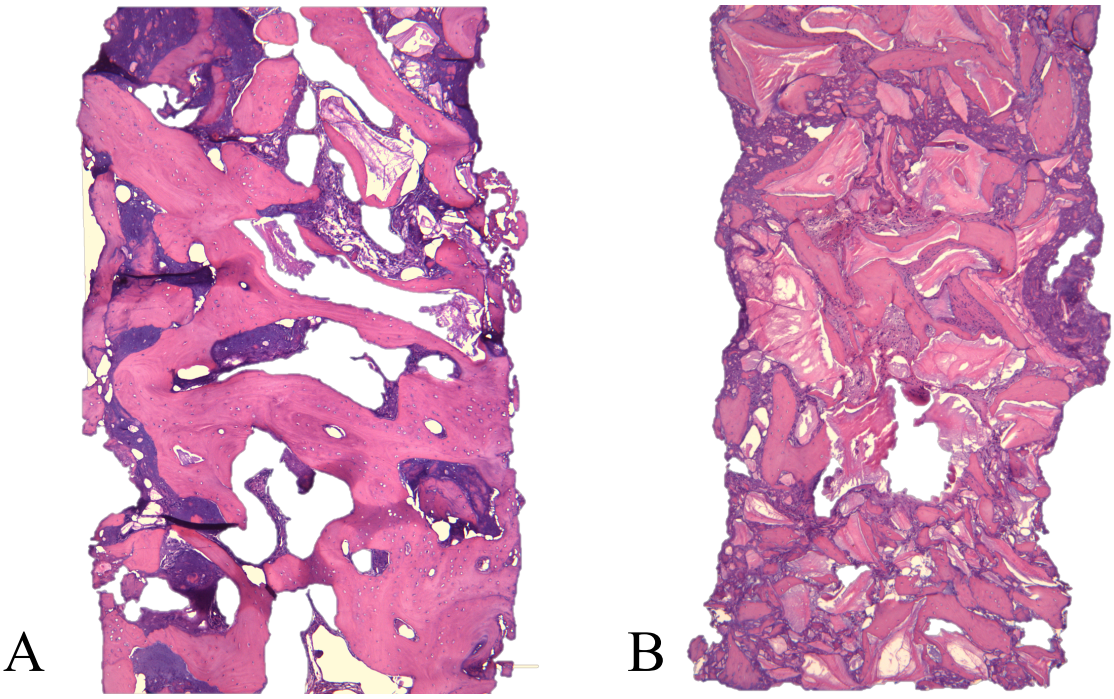


Figura 03. Imagens histológicas obtida de biópsias das áreas enxertadas de um único paciente bilateralmente (H & E 5x)

A: Imagem histológica do grupo teste do subgrupo 2 (8 meses de cicatrização).

B: Imagem histológica do grupo controle de subgrupo 2 (8 meses de cicatrização).



Fonte: Elaboração própria

A.8 Avaliação tomográfica

Todos os pacientes fizeram exame tomográfico inicial para planejamento do procedimento cirúrgico (T-0), logo após a regeneração óssea (T-1), após 4 meses para o grupo teste do subgrupo 1 e 8 meses para o grupo teste do subgrupo 2 e para o grupo controle de ambos subgrupos (T-2). Foi utilizado o tomógrafo SCANORA® 3Dx (Soredex, Tuusula, Finlândia), com uma resolução de 512 pixels, num total de 14 bits por pixels, com escala cromática de 16.384 tons de cinza.

A justificativa da quantidade de exames tomográficos se dá por meio do estudo publicado pela Associação Europeia de Osseointegração[52] em 2012.

As imagens foram processadas em formato DICOM e reconstruídas em três dimensões usando o software Planmeca. A partir dos cortes sagital foi delimitada a área enxertada corte a corte usando a codificação de cor por hiperdensidade diferencial das imagens. Após o processo de reconstrução, as dimensões volumétricas foram calculadas automaticamente no programa e representadas em centímetros cúbicos. Essa análise foi feita em todos os exames tomográficos dos pacientes de maneira cega para o analista.

Os dados obtidos foram submetidos ao teste de normalidade Teste D'Agostino & Pearson, sendo os dados com distribuição normal foram comparados pelo Teste T (teste pareado). Os dados com distribuição não normal foram analisados pelo Teste de Wilcoxon (teste não paramétrico).

ANEXO A – Comitê de Ética em Pesquisa

FACULDADE DE ODONTOLOGIA DE ARARAQUARA - UNESP

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: INFLUÊNCIA DA FIBRINA RICA EM PLAQUETAS E LEUCÓCITOS (L-PRF) NA REGENERAÇÃO ÓSSEA APÓS CIRURGIA DE LEVANTAMENTO DE SEIO MAXILAR COM BIO-OSS®: ESTUDO CLÍNICO RANDOMIZADO

Pesquisador: Daniela Leal Zandim-Barcelos

Área Temática:

Versão: 5

CAAE: 41357514.5.0000.5416

Instituição Proponente: Faculdade de Odontologia de Araraquara - UNESP

Patrocinador Principal: Financiamento Próprio
Faculdade de Odontologia de Araraquara - UNESP
FUNDACAO DE AMPARO A PESQUISA DO ESTADO DE SAO PAULO

DADOS DO PARECER

Número do Parecer: 1.375.162

Apresentação do Projeto:

Diversas técnicas de enxertia óssea têm sido propostas na literatura para restabelecimento da espessura e altura óssea de rebordos atrésicos. Um substituto ósseo de origem bovina conhecido como Bio-Oss® tem demonstrado resultados favoráveis e seguros para regeneração óssea nos procedimentos de levantamento de seio maxilar.

Porém, o período de maturação óssea deste biomaterial osteocondutora é de aproximadamente 8 meses. Com o intuito de diminuir o tempo de cicatrização dos enxertos, diferentes fatores de crescimento têm sido investigados.

Objetivo da Pesquisa:

Avaliar o potencial da fibrina rica em plaquetas e leucócitos (L-PRF) em acelerar a regeneração óssea quando usado em combinação com Bio-Oss® na região posterior de maxila após procedimentos cirúrgicos de elevação da membrana do seio maxilar.

Avaliação dos Riscos e Benefícios:

Benefícios: Bio-Oss®, amplamente estudado e testado em diversas pesquisas científicas. Este material é vendido comercialmente e demonstra resultados bastante favoráveis neste tipo de

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cirurgia. Muitos estudos mostram que este material de origem bovina se transforma em osso humano e não causa inflamação, podendo ser usado com segurança em qualquer pessoa, estando entre os materiais mais indicados para enxerto de osso na boca. O uso deste material evita a necessidade de uma segunda cirurgia para obtenção de osso do próprio paciente.
Risco: Como toda cirurgia em que ocorre incisão (corte), existe um risco potencial de hemorragia, embora isto seja muito pouco comum para este tipo de cirurgia. Pode haver inchaço e, em casos raros, falta de integração do enxerto, reações inflamatórias e/ou infecciosas no material de enxerto, ao anestésico local ou à medicação prescrita após a cirurgia.

Comentários e Considerações sobre a Pesquisa:
Pesquisa de importância para a área pois com os resultados obtidos deste estudo, poderá ser verificada se a L-PRF tem realmente capacidade de acelerar a regeneração óssea quando associada a um material osteocondutor em procedimentos de levantamento de seio maxilar. Além de determinar se o L-PRF fornece algum benefício adicional no protocolo convencional de cicatrização com utilização de Bio-Oss e será avaliada a taxa de sucesso dos implantes instalados nestas áreas enxertadas

Considerações sobre os Termos de apresentação obrigatória:
Todos os termos foram devidamente apresentados.

Recomendações:

Conclusões ou Pendências e Lista de Inadequações:
Todas as solicitações foram atendidas.

Considerações Finais a critério do CEP:
Atendidas pendências de reunião, considero APROVADA a ememda.
O pesquisador deverá encaminhar relatórios parciais a cada 01 (um) ano até o prazo final da pesquisa, quando deverá encaminhar o relatório final.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_625882_E1.pdf	14/12/2015 17:33:12		Aceito
Outros	Respostaparecerista.pdf	14/12/2015 17:31:16	Daniela Leal Zandim-Barcelos	Aceito

FACULDADE DE ODONTOLOGIA DE ARARAQUARA - UNESP

Continuação do Parecer: 1.375.162

TCLE / Termos de Assentimento / Justificativa de Ausência	TCLEemenda14122015.pdf	14/12/2015 17:27:01	Daniela Leal Zandim-Barcelos	Aceito
Folha de Rosto	FolhaRostoEMENDA.pdf	19/11/2015 14:31:17	Daniela Leal Zandim-Barcelos	Aceito
Cronograma	CronogramaEMENDA.pdf	19/11/2015 10:51:08	Daniela Leal Zandim-Barcelos	Aceito
Projeto Detalhado / Brochura Investigador	ProjetoEMENDAFINALCEP.pdf	19/11/2015 10:50:46	Daniela Leal Zandim-Barcelos	Aceito
Outros	CartaencaminamentoEMENDA.pdf	19/11/2015 10:49:36	Daniela Leal Zandim-Barcelos	Aceito
Outros	Carta cumprimento normas comite.pdf	10/12/2014 11:01:02		Aceito
Outros	Carta autorizacao laboratório Dani.pdf	10/12/2014 11:00:42		Aceito
Outros	Carta autorizacao clinica.pdf	10/12/2014 11:00:25		Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

ARARAQUARA, 18 de Dezembro de 2015

Assinado por:
Andréa Gonçalves
(Coordenador)

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ANEXO B - TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Influência da fibrina rica em plaquetas e leucócitos na formação óssea após cirurgia de elevação do assoalho do seio maxilar com osso bovino desproteinizado: estudo clínico randomizado

Por este instrumento particular, declaro, para os devidos fins éticos e legais, que eu (nome) _____, (nacionalidade)_____,(profissão)_____, portador do R.G. _____, C.I.C._____, residente à Rua/ Av. _____, na cidade de _____, Estado de _____, concordo em participar da pesquisa intitulada: "Influência da fibrina rica em plaquetas e leucócitos na regeneração óssea após cirurgia de levantamento de seio maxilar com Bio-Oss®: estudo clínico randomizado". Declaro que fui plenamente esclarecido pelo(s) pesquisador(es) sobre:

1) Esta pesquisa irá avaliar se uma substância derivada do sangue do próprio paciente, chamada de fibrina (L-PRF), é capaz de acelerar o processo de formação de osso quando usada juntamente com um substituto de osso nas cirurgias realizadas para aumentar volume de osso. Para atingir este objetivo, serão selecionados pacientes que precisam realizar enxerto de osso em ambos os lados da região posterior da maxila (osso que sustenta os dentes superiores) antes da colocação de implantes dentários.

2) Na região posterior da maxila a ausência de osso é um problema frequentemente encontrado em pacientes que decidem realizar a colocação de implantes. O volume de osso nessa região é limitado pela presença do seio maxilar (cavidade que fica acima dos dentes superiores posteriores na face) e pela perda de osso que ocorre após perda dos dentes naturais. Assim, os tratamentos com enxertos na região do seio maxilar passaram a ser muito usados na odontologia, sendo considerados procedimentos seguros. O objetivo destes procedimentos é aumentar o volume de osso e permitir a instalação dos implantes. Nestas cirurgias podem ser utilizados enxertos de osso retirados do próprio paciente (osso autógeno) e/ou substitutos de osso. Estou ciente que para participar dessa pesquisa serei submetido a cirurgia de enxerto de osso no seio maxilar para posterior instalação de implantes. Declaro que esta cirurgia de enxerto no seio maxilar é uma intervenção necessária para colocação dos implantes independente da minha participação neste estudo.

3) Estou ciente que será utilizado neste estudo um substituto de osso desproteinizado, ou seja, um osso sem tecido vivo, "seco" e "esterilizado", conhecido como Bio-Oss®, amplamente estudado e testado em diversas pesquisas científicas. Este material é vendido comercialmente e demonstra resultados bastante favoráveis neste tipo de cirurgia. Muitos estudos mostram que este material de origem bovina se transforma em osso humano e não causa inflamação, podendo ser usado com segurança em qualquer pessoa, estando entre os materiais mais indicados para enxerto de osso na boca. O uso deste material evita a necessidade de uma segunda cirurgia para obtenção de osso do próprio paciente. Entendo que remover osso de outras regiões do meu corpo apresenta uma série de limitações e desvantagens, principalmente pela necessidade de se fazer uma cirurgia em outra região e por ter um pós-operatório mais complicado. Em um lado da maxila o Bio-Oss® será utilizado sozinho e no outro lado será associado à fibrina derivada do sangue do paciente. Como esta fibrina é preparada com utilização do próprio sangue do paciente, não há riscos de infecção cruzada ou rejeição. O paciente não será informado sobre o lado da maxila em que a fibrina foi utilizada para que não haja interferência nas avaliações do

estudo.

4) Estou ciente que também será usada uma membrana reabsorvível (Bio-Gide®) para proteger e facilitar a formação de osso. Esta membrana é formada de colágeno derivado de suínos, que é processado e esterilizado. Assim como o enxerto de origem bovina, a suína também se integra ao tecido gengival e ósseo do próprio paciente formando um novo tecido na região, não causa inflamação e pode ser utilizado seguramente em qualquer pessoa como mostram diversos estudos. Apesar de muito raro, pode ocorrerum processo alérgico relacionado ao colágeno presente nos enxertos, mas sem complicações graves.

5) Além do exame clínico, serão realizadas tomografias para planejamento da cirurgia e para avaliar o volume de osso obtido imediatamente após a cirurgia e antes da colocação dos implantes. Fui informado que a tomografia que irei realizar libera uma quantidade muito menor de radiação quando comparada às tomografias médicas, e que terei que utilizar avental de chumbo e colar de tireoide para realização das tomografias.

6) A colocação dos implantes será realizada após 4 ou 8 meses da cirurgia de enxerto para o lado que recebeu Bio-Oss® e a fibrina e após 8 meses da cirurgia de enxerto para o lado que recebeu apenas Bio-Oss®. Durante a cirurgia para colocação dos implantes, será obtido um pequeno fragmento do osso que foi formado. Estou ciente que a remoção do fragmento de osso será realizada antes da colocação dos implantes. Nesta etapa, a broca utilizada para fazer a perfuração inicial do osso será substituída por uma broca perfurada no meio permitindo a coleta do fragmento de osso. A utilização desta broca não proporcionará nenhum tipo de risco adicional ou prejuízo ao meu tratamento e/ou a minha saúde.

7) Estou ciente que as cirurgias para formação de osso e para colocação dos implantes serão realizadas por pesquisadores treinados e experientes para estes procedimentos. Fui informado sobre a possibilidade de ocorrer problemas durante ou após a cirurgia. Como toda cirurgia em que ocorre incisão (corte), existe um risco potencial de hemorragia, embora isto seja muito pouco comum para este tipo de cirurgia. Pode haver inchaço e, em casos raros, falta de integração do enxerto, reações inflamatórias e/ou infecciosas no material de enxerto, ao anestésico local ou à medicação prescrita após a cirurgia. Fui esclarecido que caso ocorra perda dos enxertos ou dos implantes, os pesquisadores executarão uma nova cirurgia para repor os mesmos sem custos adicionais. Entretanto eu devo comparecer a todas as visitas agendadas e as consultas de manutenção, durante todo período da pesquisa. A medicação será prescrita pelos pesquisadores conforme indicação de uso para cada paciente, e normalmente consiste de um medicamento anti-inflamatório e analgésico (para dor, inchaço e inflamação), e um antibiótico (para prevenir infecções - bactérias). Receberei assistência total e imediata em caso de danos decorrentes dos procedimentos cirúrgicos deste estudo.

8) A coleta de sangue será realizada por um profissional habilitado para este procedimento utilizando o sistema de coleta a vácuo. Serão coletados 4 tubos de 10 mL cada. O preparo da fibrina será realizado com utilização do sangue coletado por um dos pesquisadores que fez treinamento para este procedimento. Após a remoção da fibrina formada, os tubos serão descartados em lixo específico para material pérfuro-cortante e infectante. Não será armazenado nenhum material proveniente do sangue do paciente.

9) O pesquisador responsável pela pesquisa, bem como os demais profissionais responsáveis pelo tratamento em questão (citados abaixo como demais pesquisadores) se comprometem a dar resposta a qualquer pergunta e esclarecimento ou qualquer dúvida relacionada. Afirmo que não estou sendo forçado ou induzido a fazer parte da pesquisa devido aos materiais de enxerto, implantes ou tomografias que receberei sem custo e que participo da pesquisa por livre e espontânea vontade. Sei que posso deixar de participar da pesquisa a qualquer tempo, sem prejuízo do atendimento, cuidados e tratamento pela equipe da especialidade da FOAr-UNESP. Entendo que todas as minhas informações serão confidenciais, zelando pela minha privacidade e garantindo que minha identificação não será exposta nas conclusões ou publicações da pesquisa.

10) Os conhecimentos adquiridos, com o presente estudo, serão importantes para determinar se a fibrina

derivada do sangue do paciente pode acelerar o processo de formação óssea necessário para instalação de implantes em áreas onde a quantidade de osso não é suficiente para colocação de implantes dentários. Geralmente deve-se esperar um período de aproximadamente oito meses para instalação de implantes na maxila que foi submetida a regeneração de osso no seio maxilar.

11) Fui informado que os materiais utilizados nas cirurgias de enxerto de osso e os implantes serão fornecidos aos pacientes sem nenhum custo. Não há nenhuma remuneração para participar desta pesquisa. Os gastos com transporte diretamente relacionados à participação na pesquisa serão ressarcidos quando eu solicitar.

12) Após período de cicatrização, será feita reabilitação protética dos implantes. Os componentes protéticos serão fornecidos pelos pesquisadores sem nenhum custo, porém as próteses deverão ser pagas pelos próprios pacientes. Elas não serão fornecidas aos participantes do estudo. Após instalação das próteses, todos os pacientes serão acompanhados pelo período de 2 anos para determinar o sucesso dos implantes instalados nas áreas enxertadas. Para determinação do sucesso, os implantes serão avaliados a cada 6 meses por meio de exames clínico e radiográfico. Estes exames serão realizados pelos próprios pesquisadores sem nenhum custo adicional para os pacientes. Após término do projeto, os pacientes serão encaminhados para o Projeto de Extensão “Manutenção de Pacientes Tratados com Implantes Osseointegrados” do Departamento de Diagnóstico e Cirurgia desta faculdade para tratamento e/ou manutenção em longo prazo de seus implantes.

13) Fui informado que receberei uma via do TCLE assinado por mim e pelo pesquisador.

14) Desta forma, uma vez tendo lido e entendido tais esclarecimentos, dato e assino esse termo de consentimento, por estar de pleno acordo com o teor do mesmo.

Nome da responsável pela pesquisa: Profa. Dra. Daniela Leal Zandim-Barcelos

Nome dos demais pesquisadores envolvidos na pesquisa:

Prof. Dr. Elcio Marcantonio Junior

Elton Carlos Pichotano

Cláudio Marcantonio

Telefone para contato: 3301-6376 Telefone do Comitê de Ética: 3301-6434/3301-6432

_____ Assinatura do profissional responsável

_____ Assinatura do paciente

Araraquara, ____/____/____

ANEXO C – Submissão da Publicação 1 para Clinical Oral Implants Research

CLINICAL ORAL IMPLANTS RESEARCH WILEY

Influence of leukocyte and platelet-rich fibrin on volumetric dimensional changes after maxillary sinus augmentation with deproteinized bovine bone mineral: a randomized-controlled clinical study

Journal:	*Clinical Oral Implants Research*
Manuscript ID	COIR-Nov-17-OR-6562
Manuscript Type:	Original Research
Date Submitted by the Author:	08-Nov-2017
Complete List of Authors:	Pichotano, Elton; São Paulo State University (Unesp), School of Dentistry, Diagnosis and Surgery Marcantonio Junior, Elcio; UNESP, Diagnostico e Cirurgia Grigoriadis, Agamemnon; King\'s College London Dental Institute, Centre for Craniofacial and Regenerative Biology Volponi, Ana; King\'s College London Dental Institute, Centre for Craniofacial and Regenerative Biology Austin, Rupert; King\'s College London Dental Institute, Tissue Engineering and Biophotontics Zandim-Barcelos, Daniela; Universidade Estadual Paulista Julio de Mesquita Filho, Diagnosis and Surgery; São Paulo State University (Unesp), School of Dentistry, Diagnosis and Surgery
Keywords:	Bone substitutes, Clinical research, Clinical trials, Biomaterials

ANEXO D – Submissão da Publicação 4 para Journal of Oral Implantology

Journal of Oral Implantology

Early placement of dental implants in maxillary sinus grafted with leukocyte and platelet-rich fibrin (L-PRF) and deproteinized bovine bone mineral

--Manuscript Draft--

Manuscript Number:	aaid-joi-D-17-00220R1
Full Title:	Early placement of dental implants in maxillary sinus grafted with leukocyte and platelet-rich fibrin (L-PRF) and deproteinized bovine bone mineral
Short Title:	L-PRF and maxillary sinus augmentation
Article Type:	Case Report
Keywords:	Alveolar Bone; Cone-Beam Computed Tomography; platelet-rich plasma; Sinus Floor Augmentation; dental implants.
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	Ricardo Violante de Souza, Ph.D
	Elcio Marcantonio Jr, Ph.D
	Daniela Leal Zandim-Barcelos, Ph.D
Order of Authors Secondary Information:	
Abstract:	This case report aimed to describe the effects of leukocyte and platelet-rich fibrin (L-PRF) associated with deproteinized bovine bone mineral (DBBM) and absorbable collagen membrane (CM) on bone regeneration in maxillary sinus augmentation. A 59-year-old male patient was referred to the Department of Periodontology for implant rehabilitation of his edentulous upper jaw. The treatment plan involved maxillary sinus augmentation followed by implants installation. A split-mouth design was employed, in which the right maxillary sinus was filled using L-PRF, DBBM, and CM; the left side was filled with DBBM and CM. After four and eight-months post-operatively, two dental implants were installed in each of the right and left maxillary sinus, respectively. Cone-beam computed tomography (CBCT) was taken before and after sinus augmentation for evaluation of tridimensional bone volume alterations. Bone biopsies were harvested from the implant sites for histomorphometric evaluation. Resonance frequency analysis was employed immediately after implant placement and before prosthetic rehabilitation for evaluation of implant stability. Implants were loaded 10-months after sinus augmentation. CBCT analysis showed a higher resorption rate in the right side of the maxillary sinus (L-PRF + DBBM) compared to the left side (22.25% and 8,95%, respectively). Implant stability quotient were above 68 in all time-points for both groups. Histomorphometric analysis showed highly amount of newly formed bone when L-PRF was used compared with DBBM alone (2118102 and 975535 mm3, respectively). Taken together, both techniques were effective for maxillary sinus augmentation, however the addition of L-PRF to the graft allowed early implant placement and accelerated bone healing in the conditions studied.

Araraquara, 07 de março de 2018.

Elton Carlos Pichotano