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# AVALIAÇÃO DA DEPOSIÇÃO ÓSSEA FRENTE A DIFERENTES TIPOS DE ENXERTIA COM BIOMATERIAIS EM DEFEITO CRÍTICO EM CALOTA CRANIANA DE ANIMAIS

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Tese apresentada ao Programa de Pós-Graduação em Odontologia da Universidade Estadual de Ponta Grossa, na área de Clínica Integrada, como requisito para a obtenção do título de doutor em Odontologia.

Orientador: Prof. Dr. Gilson Cesar Nobre Franco
Co-orientadora: Prof. Dra. Marcela Claudino da Silva Nardino

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Ponta Grossa, 08 de março de 2022.

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*Salmo 126: 3*

## DADOS CURRICULARES

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## RESUMO

Mecca, L.E.A. **Avaliação da deposição óssea frente a diferentes tipos de enxertia com biomateriais em defeito crítico em calota craniana de animais**. [Tese] Doutorado em Clínica Integrada. Ponta Grossa: Universidade Estadual de Ponta Grossa; 2022.

A saúde bucal é uma parte fundamental da saúde geral e colabora diretamente para qualidade de vida do paciente. Embora o edentulismo não seja uma ameaça à vida, possui impacto relevante no bem-estar do indivíduo. Com o intuito de manter a saúde dos pacientes que sofreram perdas dentárias, é da competência do cirurgião dentista prezar pela conservação de estruturas ósseas remanescentes, para que estas suportem a reabilitação protética. Apesar deste tecido apresentar capacidade reparativa, existem situações clínicas como ausência dentária por períodos prolongados, traumas e ressecções tumorais, em que estes processos naturais do osso requerem o uso de substitutos ósseos / materiais de reparo ósseo. O uso de biomateriais vêm sendo largamente utilizado para a reabilitação de defeitos ósseos de grande extensão, sendo que a escolha deste depende da sua biocompatibilidade, biodegradabilidade e a velocidade de degradação, bem como de outros fatores importantes para o sucesso da neoformação óssea do local. Considerando a relevância da aplicabilidade dos enxertos autógeno, xenógenos e aloplásticos nos procedimentos de enxertia, o objetivo destes estudos foram avaliar o processo de reparo na presença de diferentes tipos de biomateriais, em diferentes tempos cirúrgicos, em defeitos ósseos críticos na calvária de ratos e de coelhos. Sendo o primeiro experimento descrito, a comparação entre biomaterial xenógeno e biomaterial aloplástico. O segundo, apresentar diferentes possibilidades de fixação de enxertos autógenos. O terceiro apresentar o uso de biomaterial xenógeno associado a membranas de colágeno, como possibilidade de regeneração óssea guiada. Estas pesquisas têm como finalidade buscar respostas frente às diferentes características dos biomateriais e como se portam quando colocados em defeitos críticos no receptor, e apresentar prognósticos favoráveis para proporcionar qualidade de vida aos pacientes.



**Palavras-chave:** Remodelação óssea, Biomaterial, Enxerto ósseo

## ABSTRACT

Mecca, L.E.A. **Evaluation of bone deposition under different types of grafting with biomaterials in critical defect in the skull of animals.** [Tese] Doutorado em Clínica Integrada. Ponta Grossa: Universidade Estadual de Ponta Grossa; 2022.

Oral health is a fundamental part of general health and directly contributes to the patient's quality of life. Although edentulism is not life threatening, it has a relevant impact on the individual's well-being. In order to maintain the health of patients who have suffered tooth loss, it is the responsibility of the dental surgeon to care for the conservation of remaining bone structures, so that they support prosthetic rehabilitation. Although this tissue has repair and regenerative capacity, there are clinical situations such as tooth absence for prolonged periods, trauma and tumor resections, in which these natural bone processes require the use of bone substitutes/bone regeneration materials. The use of biomaterials has been widely used for the rehabilitation of bone defects of great extension, and the choice of this one depends on its biocompatibility, biodegradability and degradation rate, as well as other important factors for the success of regeneration. Considering the relevance of the applicability of autogenous, xenogeneic and alloplastic grafts in grafting procedures, the aim of these studies was to evaluate the repair process in the presence of different types of biomaterials, at different surgical times, in critical bone defects in rat and rat calvaria. rabbits. Being the first experiment described, the comparison between xenogenous biomaterial and alloplastic biomaterial. The second, to present different possibilities of fixation of autogenous grafts. The third presents the use of xenogeneic biomaterial associated with collagen membranes, as a possibility of guided bone regeneration. These researches aim to seek answers to the different characteristics of biomaterials and how they behave when placed in critical defects in the receptor, and to present favorable prognosis to provide quality of life to patients.



**Keywords:** Bone formation, Biomaterials, Bone graft.

**SUMÁRIO**

## 1 INTRODUÇÃO

A saúde bucal é uma parte fundamental da saúde geral e também considerada essencial para a qualidade do paciente (Cunha *et al.*, 2015). Embora o edentulismo não seja uma ameaça a vida, possui grande impacto no bem-estar do indivíduo (Wu, Bei *et al*., 2013), visto que influencia na disfunção do sistema estomatognático, dificultando a mastigação e fonação, podendo causar danos estéticos e psicológicas (Musacchio *et al.*, 2007; Emami *et al.*, 2013). Com o intuito de manter a saúde dos pacientes que sofreram perdas dentárias, é da competência do cirurgião dentista, prezar pela conservação de estruturas ósseas remanescentes, para que estas suportem a reabilitação protética (Pan *et al.*, 2010).

O tecido ósseo pode ser considerado altamente ativo, pois apresenta uma contínua capacidade de mineralização, remodelação e reparo. Estas características estão intimamente ligadas com o processo de homeostase do organismo humano, além de auxiliar na sustentação e proteção do mesmo (Knapik *et al.*, 2014). No entanto, apesar deste tecido apresentar capacidade reparativa, existem situações clínicas como ausência dentária por períodos prolongados, traumas ou ressecções tumorais, (Miron; Zhang, 2012) em que estes processos naturais do osso, requerem o uso de substitutos ósseos ou materiais de regeneração óssea (Yahav *et al.*, 2020).

O uso de biomateriais vem sendo largamente utilizados para a reabilitação de defeitos ósseos de grande extensão, sendo considerado como padrão ouro o enxerto autógeno, originado do próprio indivíduo. No entanto, a necessidade de uma área cirúrgica adicional, a reabsorção do enxerto e o aumento no risco de morbidade são possíveis desvantagens inerentes a este tipo de enxerto (Lutz *et al.*, 2015; Ansari, 2019). O enxerto alógeno proveniente de indivíduos da mesma espécie, apresentam propriedades osteocondutoras e osteoindutoras decorrentes da presença de proteínas morfogenéticas ósseas (BMPs) e pode ser uma opção para enxertia (Rodolfo *et al*., 2017).

Na atualidade, a aplicação dos biomateriais torna-se uma possibilidade importante aos enxertos ósseos convencionais, visto que não lesam tecidos saudáveis e não estão diretamente associados ao aumento de infecções (Brown; Laurencin,

2019). A escolha de um biomaterial depende da sua biocompatibilidade, biodegradabilidade e a velocidade de degradação, bem como de outros fatores importantes para o sucesso da regeneração (Oliveira *et al.*, 2010). Com relação a sua origem, os biomateriais podem ser de origem xenógena, ou seja, são provenientes de outras espécies, principalmente de origem bovina, sendo desproteinizados e liofilizados (Sheikh *et al.,* 2017). Este biomaterial é largamente utilizado e apresenta elevados índices de sucesso (Jensen *et al.,* 2007). Os enxertos aloplásticos representam o grupo de biomateriais quimicamente diferentes, formados à base de cálcio sintéticos, incluindo fosfato de cálcio, sulfato de cálcio, vidros bioativos e polímeros, sendo observado benéficos resultados com seu uso (Nakata *et al.*, 2016).

Atualmente, os cientistas buscam desenvolver biomateriais com algumas características apontadas como ideais, como, potencial osteocondutor, osteoindutor e que possuam características biocompatíveis, biorreabsorvíveis com integridade estrutural. As capacidades osteocondutoras, vão permitir a existência de uma matriz que possibilita o crescimento de tecido ósseo, já as propriedades osteoindutoras, possibilitam a existência de fatores que fomentam os processos de reparação e remodelação do tecido ósseo (Kačarević *et al.*, 2019).

Outras características que podem ser atribuídas a um biomaterial ideal são a versatilidade, a facilidade de ser manipulado e estabilidade imediata após a sua implantação, de forma menos traumática possível. Devem apresentar similaridade ao tecido ósseo nativo humano e estimular a distribuição de proteínas morfogénicas ósseas de forma controlada e apresentar porosidade adequada. Estes substitutos precisam apresentar um tempo de reabsorção semelhante ao tempo de regeneração do osso nativo, e ainda deve possibilitar uma boa vascularização após sua implantação (Hannink; Arts, 2011). A interação entre o material e os constituintes biológicos do receptor, não deve causar quaisquer problema a nível celular, tegumentar ou órgãos circundantes e, portanto, o material não deve ser antigénico, citotóxico, carcinogénico, pirogénico (Mathew *et al.*, 2016).

Considerando a relevância da aplicabilidade dos enxertos autógeno, xenógenos e aloplásticos nos procedimentos de enxertia, suas propriedades de reabsorção, osteoindução, osteocondução, vascularidade e diversas características

consideradas ideais para os biomateriais. O objetivo destes estudos foi avaliar o processo de reparo na presença de diferentes tipos de biomateriais, em diferentes tempos cirúrgicos, em defeitos ósseos críticos na calvária de ratos e de coelhos.

# 2 PROPOSIÇÃO

## 2.1 ARTIGO 1

Histological comparison between biphasic calcium phosphate and deproteinized bovine bone on bone defects.

### 2.1.1 Proposição geral

O objetivo deste estudo foi avaliar o processo de reparo na presença de fosfato de cálcio bifásico (BCP) e osso bovino desproteinizado (DBB), após 15 e 45 dias, em defeitos ósseos críticos na calvária de coelhos.

### 2.1.2 Proposição específica

Avaliar o padrão de deposição óssea dos grupos com e sem os diferentes tipos de biomateriais nos tempos propostos.

Estimar a histomorfometria de tecido mole, osso imaturo e biomaterial nos diferentes grupos após 15 e 45 dias.

Analisar a densidade volumétrica de fibras de colágeno, fibroblastos, vasos sanguíneos, células inflamatórias, osteoclastos e espaços em branco na área enxertada de Sham,( osso bovino desproteinizado) DBB e Grupos(fosfato de cálcio bifásico ) BCP após 15 e 45 dias.

## 2.2 ARTIGO 2

Implant osseointegration in grafted autogenous bone blocks fixed with screws and cyanoacrylate-based adhesive: histomorphometric and microCT analysis.

### 2.2.1 Proposição geral

O objetivo deste estudo foi avaliar a osseointegração de implantes em blocos ósseos autógenos enxertados, fixados com adesivo e parafusos à base de cianoacrilato.

### 2.2.2 Proposição específica

Avaliar a porcentagem de contato osso-implante e área óssea entre as roscas aos 30 e 45 dias nos grupos parafuso e adesivo.

Analisar a densidade volumétrica de osso maduro, osso imaturo, dos diferentes grupos.

Ponderar as células e componentes do tecido ósseo, nos tecidos mineralizados da interface entre o bloco ósseo e o local receptor dos grupos de parafusos e adesivos aos 60 e 75 dias dos procedimentos de enxerto.

Examinar a densidade volumétrica de fibras de colágeno, fibroblastos, vasos sanguíneos, células inflamatórias, espaços brancos, osteoblastos e adipócitos (G) nos tecidos moles de interface entre o bloco ósseo e o local receptor dos diferentes grupos.

## 2.3 ARTIGO 3

Analysis of the tissue repair of critical defects in the calotte of rats treated with particulate inorganic xenogen graft and collagen membrane.

### 2.3.1 Proposição geral

Avaliar o processo de reparo tecidual na presença de enxerto xenógeno inorgânico particulado, associado ao uso de membrana de colágeno em defeitos críticos na calota de ratos.

### 2.3.2 Proposição específica

Apreciar o processo inflamatório no leito receptor do enxerto por meio de análise histológica em defeitos críticos na calota de ratos.

Analisar a incorporação do enxerto xenógeno inorgânico particulado e da membrana de colágeno no leito receptor por meio de análise histológica em defeitos críticos na calota de ratos.

Avaliar a deposição de tecido ósseo neoformado no defeito crítico em calotas de ratos por meio de análise histológica.

# 3 ARTIGOS

## 3.1 HISTOLOGICAL COMPARISON BETWEEN BIPHASIC CALCIUM PHOSPHATE AND DEPROTEINIZED BOVINE BONE ON BONE DEFECTS.

SHORT TITLE: BONE SUBSTITUTES IN CRITICAL SIZE DEFECTS.

STATUS: ACEITO

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ABSTRACT

The limited options for bone repair have led to an extensive research of the field and the development of alloplastic and xenogeneic grafts. The purpose of this study was to evaluate bone repair with two bone substitutes: deproteinized bovine bone (DBB) and biphasic calcium phosphate ceramic (BCP) in critical size defects. Material and methods: 8-mm defects were made in the parietal bones of rabbits (n=12). The animals were divided into three experimental groups: sham (defect filled with blood clot), DBB (defect filled with DBB), and BCP (defect filled with BCP). After the experimental periods of 15 and 45 days, the animals were euthanized and submitted to histomorphometric analysis. The total defect area, mineralized tissue area, biomaterial area, and soft tissue area were evaluated. Results: a greater amount of immature bone tissue and biomaterial particles were observed in the BCP group compared to DBB and sham at 45 days ($p<0.05$). There was no difference in the qualitative pattern of bone deposition between DBB and BCP. However, the sham group did not show osteoid islands along the defect, presenting a greater amount of collagen fibers as well in relation to the DBB and BCP groups. There was a greater number of inflammatory cells in the DBB at 45 days compared to BCP and sham groups. Conclusions: BCP and DBB are options for optimizing the use of bone grafts for maxillofacial rehabilitation. Bone defects treated with BCP showed greater deposition of bone tissue at 45 days.

Keywords: Bone formation, Biomaterials, Deproteinized bovine bone, Hydroxyapatite, Bone substitutes.

# INTRODUCTION

Biomaterials have been widely used for the rehabilitation of large bone defects due to trauma, tumor resection, or long-term tooth loss. According to their origin, biomaterials can be classified as autogenous, allogeneic, xenogenous, and alloplastic (1). Autogenous bone grafts, known as the gold standard, allow the maintenance of cell viability and osteogenic capacity (1,2). However, the need for an additional surgical area, graft resorption, and increased risk of morbidity are disadvantages inherent to this type of graft (3,4).

Allogeneic grafts are harvested from donors of the same species and have osteoconductive and osteoinductive properties due to the presence of bone morphogenetic proteins (BMPs). Xenogenous grafts are harvested from other species, mainly bovine, being deproteinized and lyophilized before use (1,5,6). Xenogenous grafts are widely used biomaterials with high success rates (7,8,9). These biomaterials do not cause an immune response and are considered biocompatible. One of these materials is the Bio-Oss® (Geistlich-Pharma, Wolhusen, Switzerland), consisting of calcium carbonate apatite. It is osteoconductive, has a porosity between 75 and 80%, and can be used for maxillary sinus lift, resulting in adequate osseointegration of the dental implant (7). The material is slowly reabsorbed, and its residues can be found nine years after grafting (6,10).

Alloplastic grafts are a large group of synthetic calcium-based biomaterials of different compositions, including calcium phosphate, calcium sulfate, bioactive glasses, and polymers (5). They are widely used in the preservation of the alveolar ridge and maxillary sinus floor augmentation, with high success rates (2,7). In addition, allografts are available without quantity restriction and there is no risk of disease transmission.

Hydroxyapatite is an insoluble biphasic alloplastic biomaterial produced by the sintering of hydroxyapatite (HA) and beta-tricalcium phosphate (β-TCP). Several HA/(β-TCP) mixtures have been tested as graft materials with favorable clinical results (2,8,10,11). Studies suggest that each alloplastic material has a different resorption rate, and they can be used alone or associated with autogenous bone without altering

the osteoconductive properties (1,12).

The bone deposition on the surface of different types of biomaterials have been widely studied. Cordaro et al. (9) found no difference in new formed bone with deproteinized bovine bone (DBB) graft and β-TCP in maxillary sinus lift procedures (7,9,13). Recent results of our group demonstrated that biphasic calcium phosphate (BCP) associated to autogenous bone resulted in a greater amount of new formed bone than DBB in initial evaluations (12). In fact, each biomaterial has peculiarities regarding to characteristics and biological responses (14). In addition, the experimental model has a relevant influence on these responses (15). In this context, the evaluation of DBB and BCP in experimental models with larger animals such as rabbits are interesting and allows the creation of critical size defects in the same animal. Regarding bone metabolism, aspects such as bone composition and healing process describe the rabbit as a more suitable model (16,17).

Therefore, the encouraging findings of BCP in early bone repair associated to autogenous bone (12) led us to investigate if BCP would have similar outcomes in critical-size bone defects by itself (not associated to autogenous bone). Thus, the primary objective of this study was to evaluate qualitatively the repair process of critical bone defects in rabbit calvaria using biphasic calcium phosphate and deproteinized bovine bone. Secondary objectives were to quantitatively analyze soft and mineralized tissues after 15 and 45 days of grafting procedure using DBB and BCP.

## METHODOLOGY

A split-body experimental study was conducted using sixteen female adult New Zealand rabbits (Oryctolagus cuniculus), with mean age of 4 months, weighing approximately 3 kg. During the experimental period, the animals were kept with food and water ad libitum, at 20 °C and with 8 to 10 hours of light exposure daily. Animals underwent a 12-hour fast prior to the surgical procedure. Animals were purchased from the Central Bioterium from Pontifical Catholic University and were quarantined in pens for 14 days prior to surgical procedures for ambiance and adaptation to the surroundings.

The experimental protocol was in accordance with the World Society for the

Protection of Animals, with approval by the animal ethics committee of the institution (protocol 309/2015). During the experimental study 4 animals were lost, 2 during anesthesia and 2 in post-operative period.

The animals were allocated into three groups, according to the biomaterial used: DBB, BCP, and sham. An assistant research performed the randomization using a table of random numbers assigning each experimental unit (defect) to a treatment. In the DBB group, the defect was filled with deproteinized bovine bone (Bio-Oss®, Geistlich Pharma AG, Wolhusen, Switzerland). In the BCP group, the defect was filled by BCP alloplastic material (Maxresorb® (60% HA / 40% β-TCP) (Botiss Dental GmbH, Berlin, Germany). In groups BCP and DBB the biomaterial was inserted until the entire defect was filled. Whereas in the sham group, no material was added, and the defect was filled with blood clot only. The groups were evaluated at 15- and 45-days post-intervention. Five experimental units were used from each group at each experimental time (n=5). Assuming an average standard deviation of 5%, a two-sided test, the sample size (n=5) would provide a 90% power.

Surgical procedures

The rabbits were sedated by isoflurane inhalation and anesthetized with 10% ketamine hydrochloride (50 mg/kg) associated with 2% xylazine hydrochloride (5 mg/kg) by intramuscular injection.

After anesthesia, depilatory cream was used for fur removal of the frontoparietal region followed by vigorous asepsis using povidone-iodine. To assure trans-operative hemostasis and post-operative comfort for the animal, 0.9 mL of 2% mepivacaine with 1:200,000 norepinephrine was infiltrated in the calvaria. With a No. 10 scalpel blade, a longitudinal mucoperiosteal straight incision was performed following the sagittal suture. With the aid of a periosteal elevator, full-thickness flaps were detached, completely exposing the cortical bone of the parietal region (Figure 1A). Two bone defects were performed on the parietal bones of all rabbits, laterally to the sagittal suture, with a surgical motor and a 16:1 reduction contra angle using an 8-mm diameter surgical drill (Neodent®, Curitiba, Brazil) (Figure 1B) under abundant and continuous irrigation with saline and aspiration. The external and internal cortical bones were

removed with an Ochsenbein no. 1 micro chisel, and the dura mater was kept intact with subsequent irrigation of the cavity with saline (Figure 1C). Then, the bone defects were completely filled with one of the biomaterials or blood clot (Figure 1D). The periosteum, fascia, and aponeurosis wounds were closed with vicryl 4.0 (Ethicon, Johnson & Johnson, São Paulo, SP, Brazil) (Figure 1E), and the skin wound with 3-0 nylon suture (Ethicon, Johnson & Johnson, São Paulo, SP, Brazil) (Figure 1F).

During anesthetic induction, animals received long-acting tetracycline (1mg/kg) intramuscularly. For post-anesthetic recovery, the animals received 50 mL of glycated serum, 0.1 mg/kg of morphine for analgesia, and 1 mg/kg ketoprofen intramuscularly every 24 hours for 3 days. All surgical procedures were performed by the same operator (oral surgeon) and the same assistant (oral surgeon).

During the entire postoperative period, the animals were kept in pens provided with food and water ad libitum and evaluated daily for surgical wound condition and general health. Animals were housed with a maximum of 4 rabbits per pen before and after surgical procedures. Pre, trans and post-operative practices were developed under the supervision of a veterinary surgeon. During the immediate post-operative period animals remained under supervision and returned to their pen when showed relatively normal physiological characteristics. Animals were carefully evaluated during the first 24 post-operative hours and daily until euthanasia according to each experimental period (15 and 45 days). Normal eating patterns and other physiological characteristics are monitored.

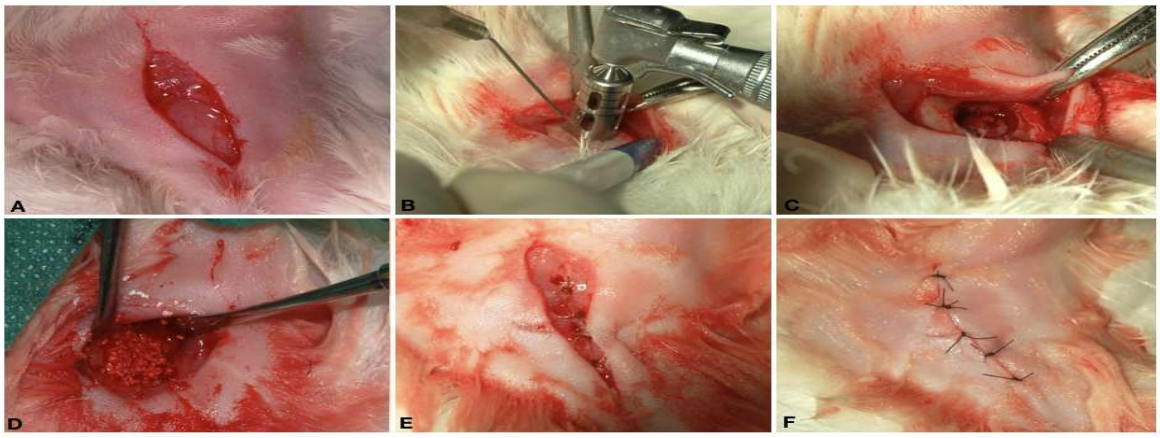


Figure - 1 schematic representation of experimental surgical procedures. The anteroposterior incision in the skullcap (a). Use of an 8mm trephine drill in the parietal bone to obtain the critical defect (b, c, and d). . Suture of the periosteum (e) and suture of aponeurotic galea fascia (f).

After 15 and 45 days, the animals were euthanized with 1g of sodium thiopental anesthetic (2mL) intravenously. The skulls were dissected, divided in half with a diamond disc, and stored in 10% formaldehyde for 48 hours, and subsequently subjected to decalcification in 10% EDTA solution (pH 7.4) for approximately 12 weeks. Then, gradual dehydration in ethanol was carried out, followed by xylene washing and paraffin embedding. Semi-serial sections 4- to 6-µm thick with a 10 µm interval were obtained and stained with hematoxylin and eosin. In all sections a random numerical sequence was assigned to codify the experimental periods and groups throughout the analysis and reduce bias. Histological evaluations were performed by a calibrated examiner.

After 15 and 45 days, there was an increase in the amount of immature bone inside the defect in the BCP group compared to DBB group. The presence of biomaterial was observed in the DBB and BCP groups in both experimental periods. In the DBB group, a significant reduction in the amount of biomaterial was observed after 45 days compared to 15 days. There was no significant difference in the amount of biomaterial between 15 and 45 days in the BCP group ($p=0.9998$), as illustrated in figures 3 and 4. The soft tissue of the DBB and BCP groups (15 and 45 days) presented well-organized fibrous connective tissue with deposition of collagen fibers in specific areas. The normal presence of blood vessels was also observed in all evaluated

groups. Necrotic areas in soft or mineralized tissue, multinucleated giant cells, and foreign body reaction were not observed in any group. The percentages of bone tissue, soft tissue, and biomaterial obtained through histomorphometry are described in Figure 5.

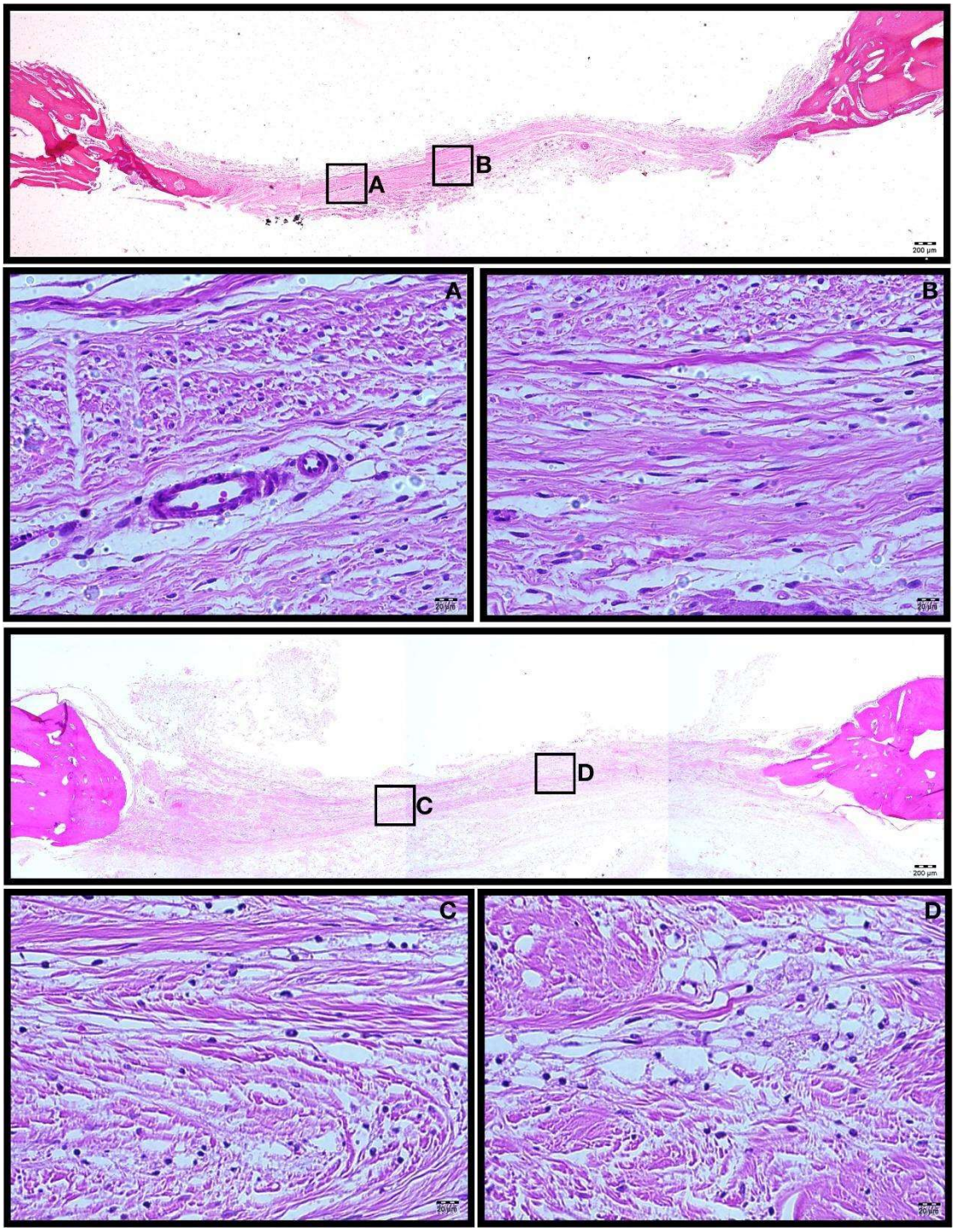


Figure 2 - bone deposition pattern of groups without the use of biomaterials (sham). Animals were evaluated for histological changes at 15 (a, b) and 45 (c, d) days after grafting procedures.

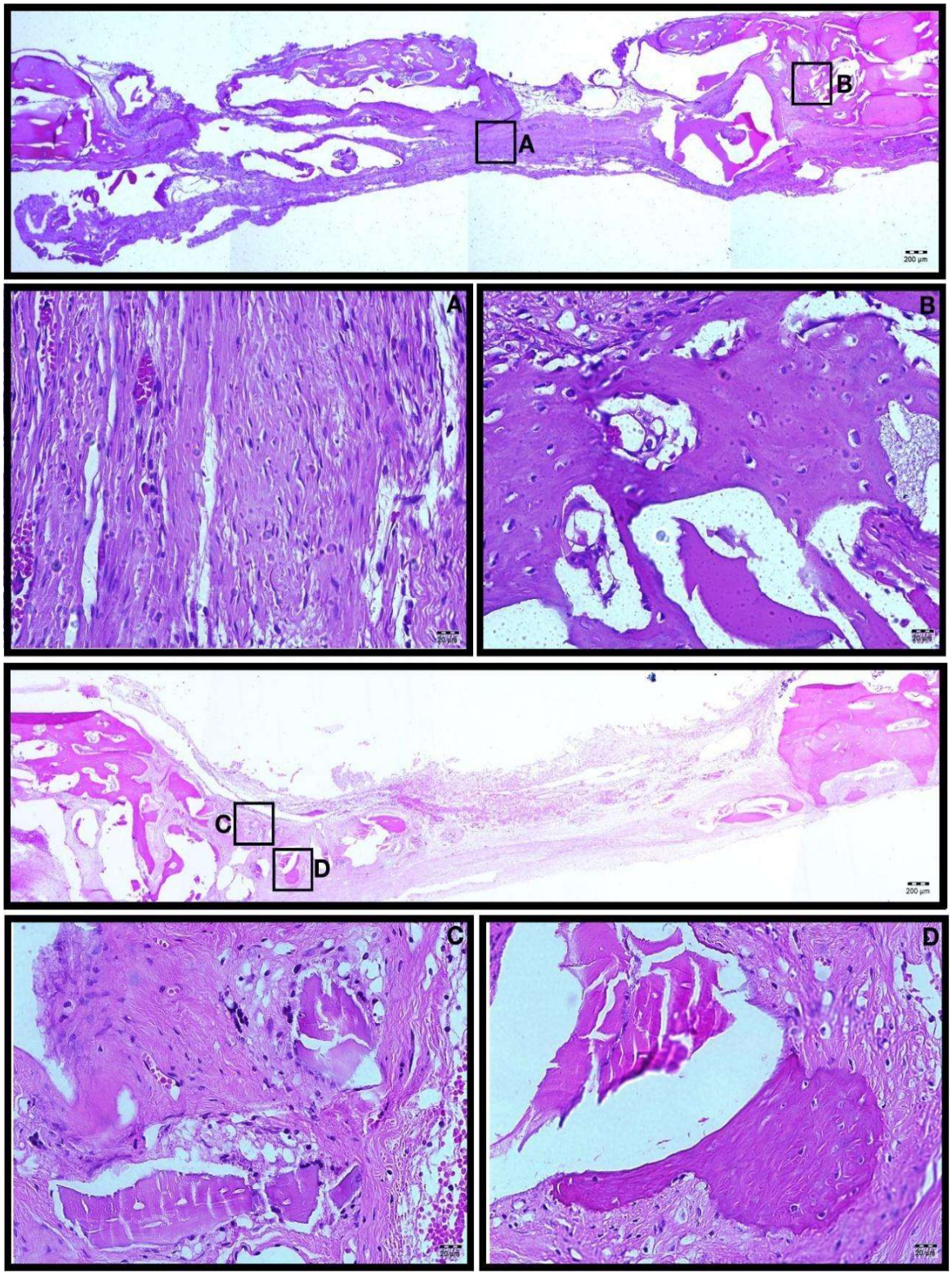


Figure 3 - bone deposition pattern of groups grafted with deproteinized bovine bone (dbb). Animals were evaluated for histological changes at 15 (a, b) and 45 (c, d) days after grafting procedures.

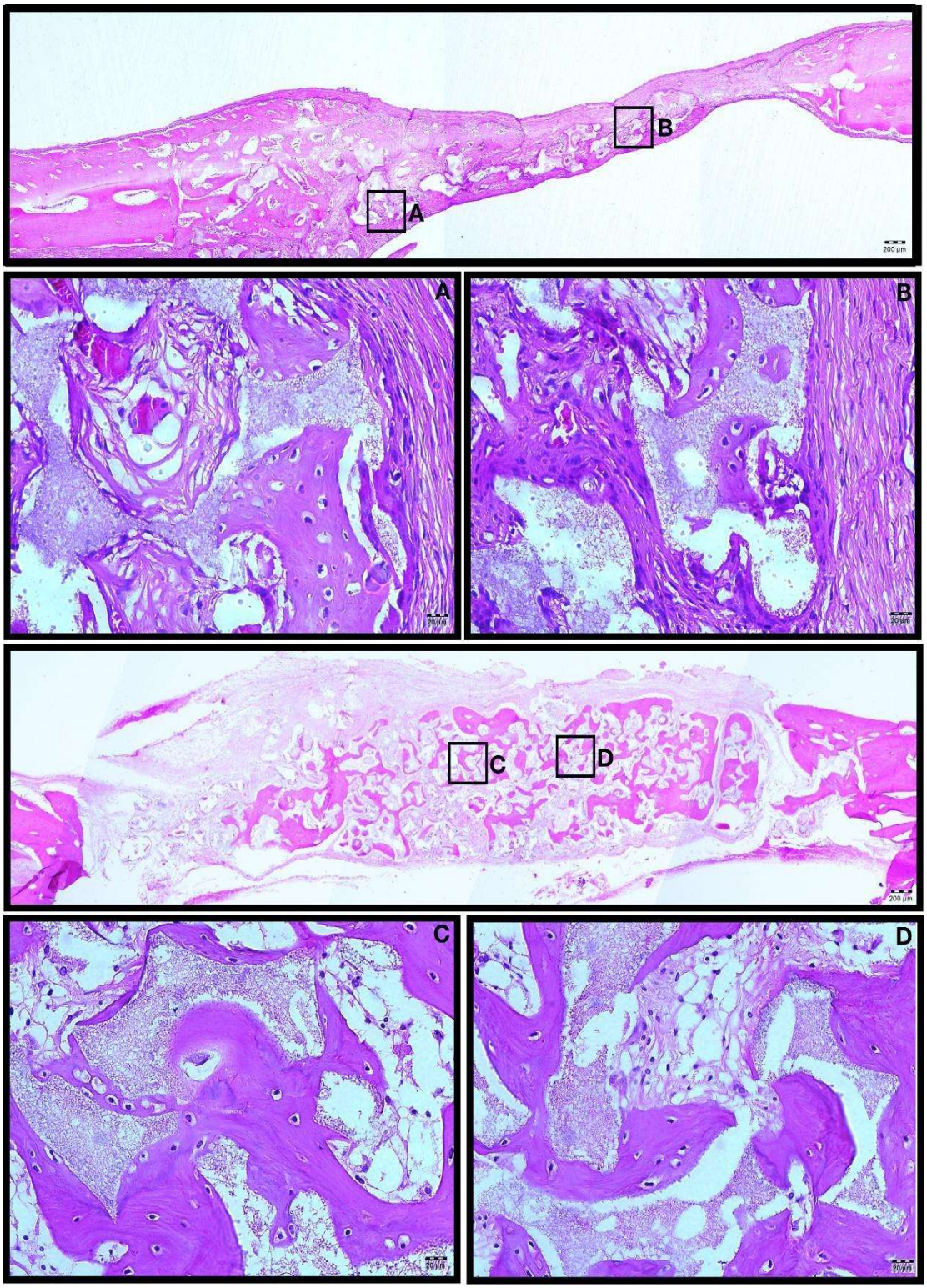


Figure 4 - bone deposition pattern of groups grafted with biphasic calcium phosphate (bcp). Animals were evaluated for histological changes at 15 (a, b) and 45 (c, d) days after grafting procedures.

The soft tissue inside the defect showed an increase in collagen fibers in the sham group after 45 days, compared to 15 days ($p<0.01$) After 45 days, DBB showed a reduction in collagen fibers in relation to the 15-day evaluation ($p<0.05$), while no variation was observed in BCP between 15 and 45 days ($p>0.05$) (Figure 6). After 15 days, BCP showed an increase of fibroblasts in relation to sham and DBB ($p<0.05$). There was no difference in fibroblast percentage between DBB and BCP after 45 days ($p=0.9999$) (Figure 6). An increase in blood vessels was observed in DBB in relation compared to sham in all experimental periods ($p<0.05$) (Figure 6). The sham group had higher inflammatory cell counts compared to DBB at 15 days ($p<0.001$). However, after 45 days, DBB inflammatory cell counts increased in relation to sham ($p<0.001$). There was no difference in inflammatory cell counts between BCP and DBB at 45 days ($p=0.5915$). A higher number of osteoclasts was found in BCP at 15 days when compared to the sham 15 ($p<0.01$), and the sham group presented small amounts of osteoclasts after 15 and 45 days (Figure 6). Blank spaces, which are usually filled by the interstitial fluid and edema, were observed in greater quantity in the DBB when compared to sham and BCP at 45 days ($p<0.05$). All results regarding to histomorphometric evaluation are presented in figures 5 and 6.

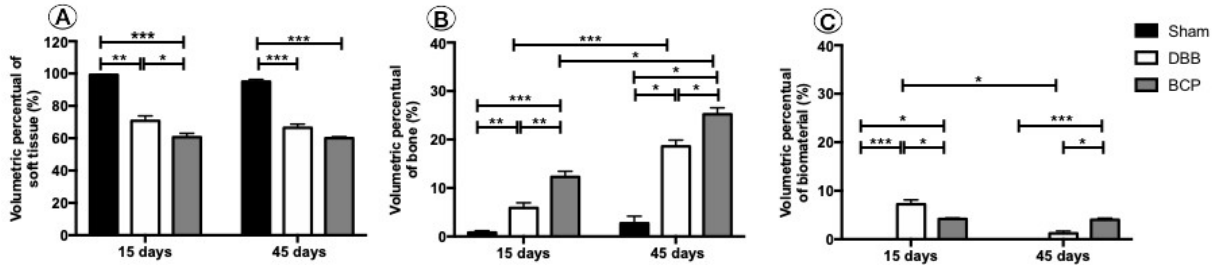


Figure 5 - histological evaluation of soft tissue (a), immature bone (b) and biomaterial (c) for sham, dbb, and bcp groups after 15 and 45 days. * indicate $p<0.05$;** indicate $p<0.001$ (twoway analysis of variance, followed by t-test).

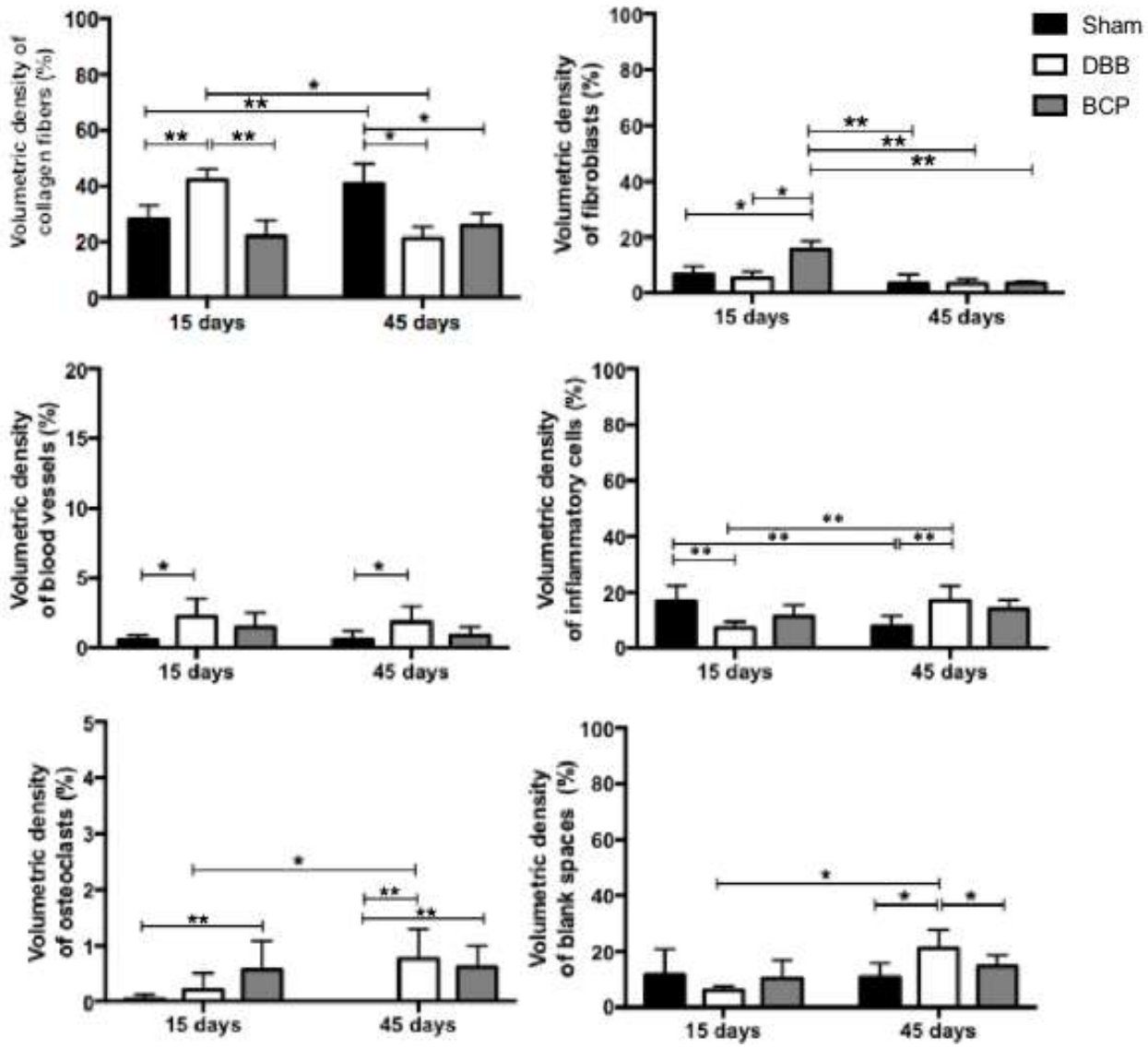


Figure 6: volumetric density of collagen fibers (a), fibroblasts (b), blood vessels (c), inflammatory cells (d), osteoclasts (e), and blank spaces (f)

## DISCUSSION

Several studies have evaluated bone deposition and tissue repair using biomaterials in different types of defects, both in humans (9,13,18,19) and in experimental models (6,10,12,20). The results of these previous studies indicate that the use of xenogenous and alloplastic biomaterials is preferred because of the reduced morbidity of grafting procedures in relation to autogenous bone. Despite extensive research in the past 30 years it is still needed to study the use of biomaterials that

meets all gold-standard requirements and desirable properties (14).

Several studies within in this area were carried out in humans, aiming at providing results with clinical applicability (7,9,13,18,19). However, experimental models allow evaluations at shorter times, the control of external factors, and the selection of different experimental periods. For such reasons, the model used in the present study has been widely applied (6,10,21). The type of defect performed in rabbits' calvaria allows an adequate interface with the biomaterial and is similar to the maxillary bone in terms of embryological origin and morphology (22). Moreover, rabbits are larger than mice or rats and their bone regeneration process is more similar to that of humans (16,17,23).

In the present study, an increase in bone deposition in DBB and BCP was observed at 15 and 45 days in relation to sham, confirming the osteoconductive ability of both biomaterials. The sham animals showed no areas of mineralized tissue inside the defect. Bone deposition in the sham group probably occurred at the edges of the defect, considering that a critical size defect was performed. Moreover, DBB and BCP had areas of mineralized tissue along the defect, closely to biomaterial particles. BCP is osteoinductive and able to stimulate the new formation of bone suggesting that it may be an alternative to treatment of large bone defect (24).

Different types of biomaterials promote different bone deposition. In our study, BCP resulted in higher bone deposition rates compared to DBB at 45 days, which corroborates the results obtained by other authors (6,10,12,25). However, similar amounts of new formed bone with the same histological characteristics have also been reported for DBB and BCP (9,20).

Perhaps, the higher bone deposition observed in the BCP group is related to the role of ceramics in osteoinduction (21,24,26), as ectopic bone formation has been observed in muscle and new bone has been found in bone sites without any contribution from osteoconduction (1). Thus, it is possible that endogenous proteins such as BMPs are absorbed and get concentrated on the ceramic surface, contributing to the attraction of osteogenic cells. In addition, the topography of ceramics and the release of inorganic ions from ceramics could also trigger the process of osteogenic differentiation and bone formation (26). Previous authors observed significantly greater

amount of new formed bone using BCP at 4 and 8 weeks when compared with other biomaterials, including DBB (10). Thus, our results together with previous ones, confirm the applicability of DBB and BCP as possible alternatives to autogenous bone grafting (25,27). Otherwise, such materials could be used in association with autogenous bone grafts (12), minimizing surgical morbidity when greater amounts of bone are needed.

The amount of biomaterial particles present in the defect was similar between 15 and 45 days for BCP and significantly reduced in DBB. However, both groups had biomaterial remnants after 45 days. Such finding is in accordance with previous studies, where no sign of DBB resorption was detected after 30 days (27,28) and DBB graft materials were not resorbed completely after 180 days (27). In fact, some authors have considered DBB a non-resorbable graft because it needs three to six years to resorb (28). Moreover, BCP reabsorption usually occurs earlier than DBB since β-TCP particles have a high resorption rate (12,21). However, some authors have not observed significant differences between DBB and BCP reabsorption after 180 days (27).

Bone formation did not occur in the defect of sham animals, while in the BCP and DBB animals bone deposition occurred in a centripetal manner, from the edges of the defect and throughout the defect, in close contact with the surface of the biomaterial. Our results corroborate those of other authors, in which BCP and DBB promoted similar bone formation (9,10,25).

Histological analysis showed a greater amount of collagen fibers in the soft tissue present throughout the defect of the sham group after 15 and 45 days when compared to DBB and BCP. This finding is probably related to the absence of new formed bone in those animals. On the other hand, the new formed bone in the groups that received the biomaterials is related to the greater presence of osteoclasts compared to the sham groups. In addition, a higher number of blood vessels, inflammatory cells, and blank spaces was observed in the DBB. Some authors also demonstrated presence of residual material, large quantity of macrophages, presence of new formed bone, blood vessels, and small amounts of collagen fibers (29) in groups treated with biomaterials. In addition, it has been demonstrated that particles of BCP

used in bone repair are surrounded by connective tissue, osteoid islands, and new formed bone (11).

The data obtained in this study emphasize the importance of using biomaterials to optimize tissue repair, since DBB and BCP showed more favorable results than the control animals. Despite the differences observed between DBB and BCP, both biomaterials can be used as alternatives to autogenous bone graft (7,25,27). Moreover, no difference was found in marginal bone of areas treated with DBB and BCP that received osseointegrated implants, neither implant stability nor differences on the survival rate (4,7). The BCP treated defects showed greater bone deposition and a greater amount of biomaterial residual in the bone defect. Previous studies have reported more active bone deposition and better remodeling of bone defects that were grafted with BCP when compared to DBB (25).

The use of an animal model is a limitation of this study and the extrapolation of these data to clinical settings must be done with caution. Further studies with other methodologies such as microtomography and molecular evaluations should be carried out to optimize treatment strategies for the rehabilitation of patients in need of bone grafting procedures. In conclusion, both biomaterials demonstrated to be suitable to critical defect grafting, however BCP resulted in greater bone deposition specially an increase in the amount of immature bone in earlier analyses.

RESUMO

As opções limitadas para reparo ósseo levaram ao desenvolvimento de abrangente pesquisa na área de enxertos aloplásticos e xenogênicos. O objetivo deste estudo foi avaliar o reparo ósseo com dois substitutos ósseos: osso bovino desproteinizado (DBB) e cerâmica fosfática de cálcio bifásica (BCP) em defeito de tamanho crítico. Material e métodos: defeitos críticos de 8 mm foram feitos nos ossos parietais de coelhos (n = 12). Os animais foram divididos em três grupos experimentais: sham (defeito preenchido com coágulo sanguíneo), DBB (defeito preenchido com DBB) e BCP (defeito preenchido com BCP). Após os períodos experimentais de 15 e 45 dias, os animais foram sacrificados e submetidos à análise histomorfométrica. Foram avaliadas a área total de defeitos, área de tecidos

mineralizados, área de biomateriais e área de tecidos moles. Resultados: maior quantidade de tecido ósseo imaturo e de partículas de biomaterial foram observados no grupo BCP em comparação aos grupos DBB e sham aos 45 dias ($p<0,05$). Não houve diferença no padrão qualitativo de deposição óssea entre DBB e BCP. Ainda, o grupo sham não apresentou ilhas osteóides ao longo do defeito, apresentando maior quantidade de fibras colágenas em relação aos grupos DBB e BCP. Houve maior quantidade de células inflamatórias no DBB aos 45 dias em comparação aos grupos BCP e sham. Conclusões: BCP e DBB são opções para otimizar o uso de enxertos ósseos na reabilitação de pacientes. Defeitos ósseos tratados com BCP mostraram maior deposição de tecido ósseo aos 45 dias.

References

1. Miron RJ, Zhang YF. Osteoinduction: a review of old concepts with new standards. J Dent Res 2012;91:736-744.

2. Danesh-Sani S, Wallace S, Movahed A, El Chaar E, Cho S, Khouly I, et al. Maxillary sinus grafting with biphasic bone ceramic or autogenous bone: clinical, histologic, and histomorphometric results from a randomized controlled clinical trial. Implant Dent 2016;25:588-593

3. Jensen SS, Yeo A, Dard M, Hunziker E, Schenk R, Buser D. Evaluation of a novel biphasic calcium phosphate in standardized bone defects. A histologic and histomorphometric study in the mandibles of minipigs. Clin Oral Impl Res 2007;18:752–760.

4. Lutz R, Berger-Fink S, Stockmann P, Neukam FW, Schlegel KA. Sinus floor augmentation with autogenous bone versus a bovine- derived xenograft – a 5-year retrospective study. Clin Oral Impl Res 2015;26:644-648

5. Misch C, Dietsh F. Bone-grafting materials in implant dentistry. Implant Dent 1993;2:158-167.

6. Takauti C, Futema F, Brito Junior R, Abrahão A, Costa C, Queiroz C. Assessment of bone healing in rabbit calvaria grafted with three different biomaterials. Braz Dent J 2014;25:379-384.

7. Oh J, Seo Y, Lee G, You J, Kim S. A comparative study with biphasic calcium phosphate to deproteinized bovine bone in maxillary sinus augmentation: a prospective randomized and controlled clinical trial. Int J Oral Maxillofac Implants 2019;34:233–242.

8. Lange GL, Overman JR, Farré-Guasch E, Korstjens CM, Hartman B, Langenbach GEJ, et al. A histomorphometric and micro-computed tomography study of bone regeneration in the maxillary sinus comparing biphasic calcium phosphate and deproteinized cancellous bovine bone in a human split-mouth model. Oral Surg Oral Med Oral Pathol Oral Radiol 2014;117:8-22.

9. Cordaro L, Bosshardt DD, Palattella P, Rao W, Serino G, Chiapasco M. Maxillary sinus grafting with Bio-Oss or Straumann Bone Ceramic: histomorphometric results from a randomized controlled multicenterclinical trial. Clin Oral Implants Res 2008;19:796-803.

10. Park J-W, Kim E-S, Jang J-H, Suh J-Y, Park K-B, Hanawa T. Healing of rabbit calvarial bone defects using biphasic calcium phosphate ceramics made of submicrosized grains with a hierarchical pore structure. Clin Oral Impl Res 2010;21;268–276.

11. Bouwman W, Bravenboer N, Frenken J, ten Bruggenkate CM, Schulten EAJM. The use of a biphasic calcium phosphate in a maxillary sinus floor elevation procedure: a clinical, radiological, histological, and histomorphometric evaluation with 9- and 12-month healing times. Int J Implant Dent 2017;3:34.

12. Uetanabaro LC, Claudino M, Zancan R, Zielak JC, Garlet GP, de Araujo MR. Osteoconductivity of biphasic calcium phosphate ceramic improves new bone formation: a histologic, histomorphometric, gene expression, and microcomputed tomography study. Int J Oral Maxillofac Implants 2020;35:70-78.

13. Froum SJ, Wallace SS, Cho SC, Elian N, Tarnow DP. Histomorphometric comparison of a biphasic bone ceramic to anorganic bovine bone for sinus augmentation: 6- to 8-month postsurgical assessment of vital bone formation. A pilot study. Int J Periodontics Restorative Dent 2008;28:273-281.

14. Haugen HJ, Lyngstadaas SP, Rossi F, Perale G. Bone grafts: which is the ideal biomaterial? J Clin Periodontol 2019;46:92-102.

15. Mardas N, Dereka X, Donos N, Dard M. Experimental model for bone regeneration

in oral and cranio-maxillo-facial surgery. J Invest Surg 2014;27:32-49.

16. Permuy M, López-Peña M, Muñoz F, González-Cantalapiedra A. Rabbit as model for osteoporosis research. J Bone Miner Metab 2019;37:573- 583.

17. Stübinger S, Dard M. The rabbit as experimental model for research in implant dentistry and related tissue regeneration. J Invest Surg 2013;26:266-282.

18. Gultekin BA, Cansiz E, Borahan O, Mangano C, Kolerman R, Mijiritsky E, et al. Evaluation of volumetric changes of augmented maxillary sinus with different bone grafting biomaterials. J Craniofac Surg 2016;27:144-148.

19. Fienitz T, Moses O, Klemm C, Happe A, Ferrari D, Kreppel M, et al. Histological and radiological evaluation of sintered and non-sintered deproteinized bovine bone substitute materials in sinus augmentation procedures. A prospective, randomized-controlled, clinical multicenter study. Clin Oral Investig 2017;21:787-794.

20. Tovar N, Jimbo R, Gangolli R, Perez L, Manne L, Yoo D, et al. Evaluation of bone response to various anorganic bovine bone xenografts: an experimental calvaria defect study. Int J Oral Maxillofac Surg
2014;43:251-260.

21. Choi H, Park N, Jamiyandorj O, Hong MH, Oh S, Park YB, et al. Improvement of osteogenic potential of biphasic calcium phosphate bone substitute coated with synthetic cell-binding peptide sequences. J Periodontal Implant Sci 2012;42:166-172.

22. Borie E, Fuentes R, Del Sol M, Oporto G, Engelke W. The influence of FDBA and autogenous bone particles on regeneration of calvaria defects in the rabbit: a pilot study. Ann Anat 2011;193:412-417.

23. Di Bella C, Farlie P, Penington A. Bone regeneration in a rabbit critical-sized skull defect using autologous adipose-derived cells. Tissue Eng Part A 2008;14:483-490.

24. Santos PS, Cestari TM, Paulin JB, Martins R, Rocha CA, Arantes RVN, et al. Osteoinductive porous biphasic calcium phosphate ceramic as an alternative to autogenous bone grafting in the treatment of mandibular bone critical-size defects. J Biomed Mater Res B Appl Biomater 2018;106:1546-1557.

25. de Lange GL, Overman JR, Farré-Guasch E, Korstjens CM, Hartman B, Langenbach GE, et al. A histomorphometric and micro-computed tomography study of

bone regeneration in the maxillary sinus comparing biphasic calcium phosphate and deproteinized cancellous bovine bone in a human split-mouth model. Oral Surg Oral Med Oral Pathol Oral Radiol 2014;117:8-22.

26. Bouler J, Pilet P, Gauthier O, Verron E. Biphasic calcium phosphate ceramics for bone reconstruction: A review of biological response. Acta Biomater 2017;53:1-12.

27. Ezirganli S, Kazancioglu HO, Mihmanli A, Sharifov R, Aydin MS. Effects of different biomaterials on augmented bone volume resorptions. Clin Oral Implants Res 2015;26:1482-1488.

28. Ezirganlı S, Polat S, Barıs E, Tatar I, Çelik HH. Comparative investigation of the effects of different materials used with a titanium barrier on new bone formation. Clin Oral Implants Res 2013;24:312–319.

29. Manfro R, Fonseca F, Bortoluzzi M, Sendyk WR. Comparative, Histological and histomorphometric analysis of three anorganic bovine xenogenous bone substitutes: bio-oss, bone-fill and gen-ox anorganic. J Maxillofac Oral Surg 2014;13:464-470.

## 3.2 IMPLANT OSSEOINTEGRATION IN GRAFTED AUTOGENOUS BONE BLOCKS FIXED WITH SCREWS AND CYANOACRYLATE-BASED ADHESIVE: HISTOMORPHOMETRIC AND MICRO CT ANALYSES.

STATUS: ACEITO

REVISTA: THE INTERNATIONAL JOURNAL OF ORAL & MAXILLOFACIAL IMPLANTS

ABSTRACT

Purpose: To evaluate implant osseointegration in grafted autogenous bone blocks fixed with cyanoacrylate-based adhesive and screws. Also, we aimed to evaluate grafted bone fixed either with adhesive or screw. Methods: Two surgical defects in the parietal region of rabbits (n=12) were performed in each animal. Autogenous bone blocks obtained were fixed anteriorly with a screw or cyanoacrylate-based adhesive. After 30 and 45 days of grafting procedures, implants were placed in bone blocks. Histomorphometric and microCT analysis of implant area were performed at 30 days after implant surgery in screw (n=6) and adhesive (n=6) groups. Histomorphometric analyses of bone-grafted areas were performed at 60 and 75 days in screw (n=6) and

adhesive (n=6) groups. Histomorphometric evaluation were carried out in implant and grafted bone areas. MicroCT parameters evaluated were BIC, BAFO, BS/BV, Tb.Th, Tb.N, Tb.Sp. Results: No differences were observed in the microCT parameters for screw and adhesive groups in all experimental periods. However, an increased quantity of the immature bone volume was observed on the adhesive group in the grafted area after 75 days. Grafted area in the screw group (75 days) presented a decrease in the volume density of the immature bone when compared to the screw group 60 days. There were no differences in both groups and experimental periods in soft tissue in the grafted area. Conclusion: no differences were observed in the osseointegration of implants placed in grafted autogenous bone blocks fixed with cyanoacrylate-based adhesive and screws. Therefore, osseointegration in autogenous bone fixed with cyanoacrylate-based adhesive is viable and may be an alternative in the near future.

Keywords: Bone transplantation, Bone remodeling, Cyanoacrylates, Dental implants, Osseointegration.

## INTRODUCTION

Teeth loss results in functional and aesthetic impairments. This absence does not allow mechanical stimulus inherent to mastication to be transmitted to the alveolar bone, resulting in reabsorption of the alveolar ridge, which significantly hinders the prosthetic rehabilitation of these patients[1].

Several prosthetic strategies are described for the rehabilitation of edentulous patients. The placement of dental implants is a safe and predictable alternative for the rehabilitation of totally or partially edentulous patients[2]. However, the success of this treatment is strictly influenced by both density and bone volume available for implant placement[3,4]. In cases of atrophic alveolar ridges, reconstruction and augmentation of severely resorbed alveolar ridges have been performed using different grafting materials and techniques in order to allow the placement of dental implants[5].

Autogenous bone is considered the gold standard in bone grafting procedures and it presents success rates of implants in grafted sites from 72.8 to 97% after follow-up periods from 6 months to 10 years[6,7]. The use of autogenous bone can be performed in block or in particulate form. The use of blocks involves fixation methods such as screws and miniplates[8,9]. Despite the frequent clinical applicability, the use of

these fixation devices has some disadvantages, including the need for removal, risk of the screw fracture during its removal or loosening, artifact formation in imaging exams, infection, and the need to remove screws that interfere with the dental implants[10]. Moreover, in regions of reduced mucosal thickness, the screws can be visible or exposed, resulting esthetic discomfort, increased risks of infection and graft loss [11].

The use of cyanoacrylate-based adhesives has been suggested as an alternative to the use of screws in the graft fixation[12-14]. In this way, cyanoacrylate-based adhesives must be biocompatible, easy to handle and slowly biodegradable to guarantee stability during the healing process[15]. Moreover, bacteriostatic and hemostatic properties have been reported[16,17]. Regarding to hemostatic properties, cyanoacrylate-based adhesives are able to quickly achieve mechanical hemostasis by creating a physical blockade to halt excessive bleeding[18]. In fact, favorable clinical results on controlling hemorrhage has been reported with cyanoacrylate glues[19]. The potential for antibacterial activity is especially associated with gram-positive microorganisms such as staphylococci and streptococci. However, some authors reported bacteriostatic activity of ethyl-cyanoacrylate for *Escherichia coli* and *Klebisiella pneumoniae.* The antibacterial effects of these adhesives are related to its short polymer chain length, which may be associated to increased degradation and release of toxic components[20,21].

Some studies have demonstrated that these cyanoacrylate-based adhesives are biocompatible and promote stability to the autogenous bone blocks similar to screws[13,14]. Conversely, other authors have reported better fixation of bone blocks with screws, associated to most extensive necrosis area in the autogenous bone blocks fixed with cyanoacrylate-based adhesives[10].

In this context, histological evaluation of bone recipient after grafting procedures seems to be interesting, since dental implants are commonly placed in these regions. In addition, the putative effects of different methods of fixation of bone autografts on osseointegration remains partially described. Therefore, the aim of this study was to evaluate implant osseointegration in grafted autogenous bone blocks fixed with cyanoacrylate-based adhesive and screws.

## MATERIAL AND METHODS

Twelve male adult rabbits, 20-22 weeks old, weighing between 2900g and 3500g were used. All the experimental procedures were approved by the Animal Ethics committee (Universidade Positivo - protocol 383/2016) and were conducted in accordance with the ARRIVE Guidelines.

### Surgical Procedures

Initially, animals were submitted to general anesthesia with inhaled isoflurane and intramuscular administration of ketamine hydrochloride 10% (40mg/kg) xylazine hydrochloride 2% (5mg/kg). An anteroposterior incision was performed on the rabbit calvaria, followed by a detachment of the soft tissue. Two surgical defects of 8mm using a trephine bur were performed under copious saline irrigation at 912rpm, in the parietal bone region, lateral to the median sagittal suture. Two bone blocks obtained were used as autogenous bone grafts and were fixed in the calvaria in the anterior region of bone defect. The fixation of the autogenous bone blocks was performed with screw or adhesive (Figure 1). In screw group (n=12), a gingival screw measuring 1.6 x 5mm (Neodent, Brazil) was fixed in the center of the bone block. In adhesive group (n=12), bone grafts were fixed with ethyl-cyanoacrylate (Super Bonder® - Loctite-Brazil). A drop was placed over the contact area using a 25-gauge needle in order to cover a half of contact area. The bone block was positioned and pressured for 20 s during adhesive polymerization.

Subsequently, periosteum, fascia and aponeurotic galea were sutured with absorbable sutures (polygalactin 910, Shalon, São Luis de Montes Belos, GO, Brazil) and skin was sutured with mononylon 3-0 (Johnson & Johnson, São Paulo, SP, Brazil). All surgical procedures were carried out by the same operator. Immediate postoperative analgesia was performed with 1 mg/mL (0.1 mg/kg) morphine and intramuscular injection of ketoprofen100mg/mL (1mg/kg) once a day for three days, and intramuscular injection of enrofloxacin 10mg/kg, once a day for seven days. Animals were kept in individual cages under the same environmental conditions before

surgery and during the evaluation period. Animal health and clinical conditions were observed during daily evaluations.

After 30 days of grafting procedures, titanium implants were placed in the grafted area using the same anesthetic and surgical parameters described above. Self-drilling anchorage implants (1.6 x 5mm) were placed in accordance with manufacturer instructions (Neodent, Neoporos surface, Curitiba, Brazil) in the central region of graft block.

After 30 and 45 days of implant animals were euthanized with $CO_2$ in concentration of up to 40%. Each specimen was clinically evaluated for absence of graft mobility. Calvarial regions were dissected and cut with a diamond disc. The specimens were stored in alcohol until microCT scanning and then samples were fixed in 10% formaldehyde solution.

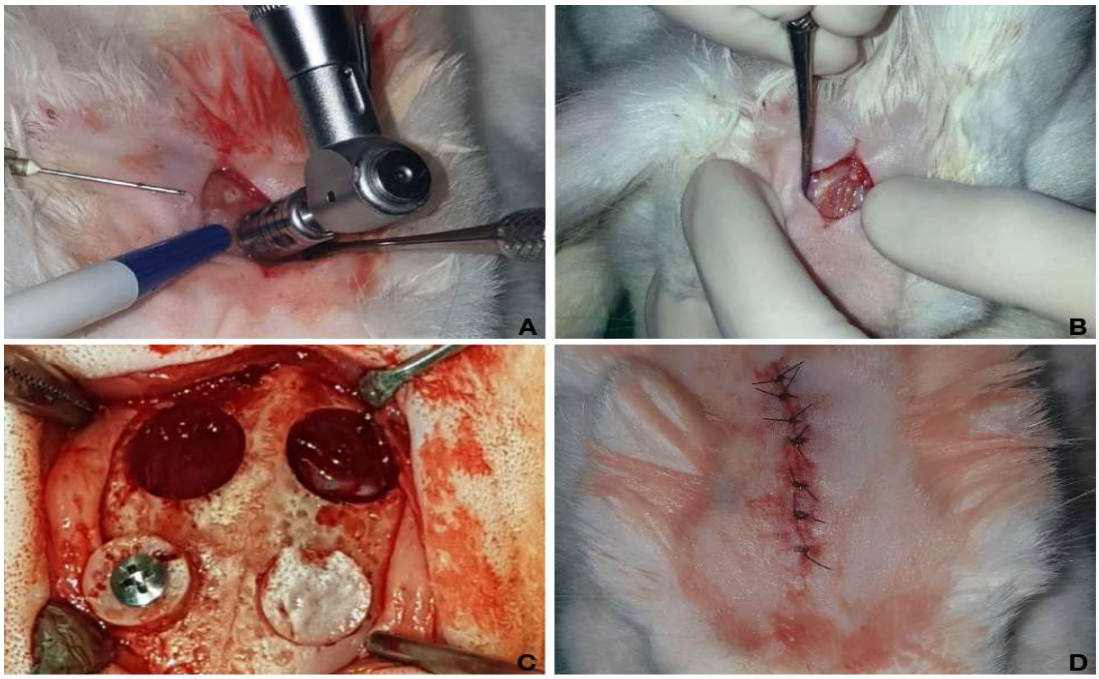


Figure 1 bone graft surgical procedures. (a) anteroposterior incision and detachment of the soft tissue on the rabbit calvaria. (b) surgical defects performed by trephine bur under copious saline irrigation. (c) autogenous bone grafts fixed in the anterior region of bone defect with screw or cyanoacrylatebased adhesive. (d) skin was sutured with mononylon.

MicroCT analysis – implant area

MicroCT data were acquired with a high-resolution microCT system (SkyScan

1272 Bruker-microCT, Kontich, Belgium) with the following parameters: energy of 90 kV, intensity of 278 mA, resolution of 9 µm pixels using a Cu 0.1 mm filter, 360° rotation, rotation step 0.2. Image reconstruction was achieved with the NRecon software (version 1.6.10.1; Bruker) calibrated by a smoothing of 1 and a ring artifact reduction of $2^{22}$.

The analyses were quantified using the CTAn version 1.14.4.1 software (Bruker) automated image analysis system. Threshold levels for bone and implant were determined for bone (max. 100, min. 43) and implant (max. 255, min. 110).

All assessments were carried out by the same calibrated examiner. The bone parameters evaluated: bone-to-implant contact (BIC, $mm^3$), bone area fraction occupancy (BAFO, %), bone volume fraction (BS/BV, 1/mm), trabecular thickness (Tb.Th, mm), trabecular separation (Tb.Sp, mm), and trabecular number (Tb.N 1/mm) were calculated for each volume of interest (VOI). The VOI was selected around the implant and defined as a column extending a total of 4mm. The height was defined by the upper and lower limits, which were 20 slices above and 20 slices under the interface of grafted bone and calvarial bone. To eliminate a possible artifact zone, the four voxels closest to the implant's surface were excluded.

Histomorphometric analysis – implant area

After fixation procedures, tissue blocks with implants were washed in running water for 24 h. These samples were dehydrated through an ethanol gradient of 70%, 80%, 90% and 100% with 7 days at 5°C. Following dehydration, the samples were embedded in a methacrylate-based resin (LR White hard grade, London Resin Company, Theale, Berkshire, UK), according to the manufacturer's instructions.

Specimens were sectioned along the longitudinal axis with a precision diamond disk (Minitom, Struers, Ballerup, Hovedstaden, Denmark), resulting in section with ~300 µm thickness. These sections were glued to acrylic plates with an acrylate-based cement. The sections were then reduced to a final thickness of ~30 µm by means of a series of SiC abrasive papers (120, 220, 320, 500, 1200 and 2000) (Struers, Ballerup, Hovedstaden, Denmark) in a grinding/polishing machine (TegraSystem, Struers,

Ballerup, Hovedstaden, Denmark) under water irrigation. The sections were submitted to toluidine blue staining and evaluated under optical microscopy[23].

The identification of histological sections was replaced by a random numerical sequence to carry out a blind evaluation in relation to the experimental periods and groups. Histomorphometric evaluation was performed using an optical microscope (Olympus BX41, Shinjuku, Japan) by a blind and calibrated examiner (LEM). Osseointegration process was evaluated throughout measurements of bone-to-implant contact (BIC) and bone area between threads (BABT) using the software Image Tool 3.0 (San Antonio Dental School, University of Texas Health Science, TX, USA).

BIC measurements were obtained by subtracting the bone-implant contact length from the total length of the threads. In BABT, we firstly obtained the total area of threads were firstly obtained and, in the sequence, the percentage of total area of threads occupied by bone tissue was determined. These assessments were performed bilaterally in the first three threads of each implant by a single calibrated examiner[23].

Histomorphometric analysis – bone-grafted areas

Bone-grafted areas, immediately after the implant, were decalcified for 8 weeks in 18% EDTA, washed, dehydrated and embedded in paraffin. Serial sections with 5 mm thickness were obtained and submitted to hematoxylin and eosin staining. Histomorphometric evaluation was performed using an optical microscope (Olympus BX41, Shinjuku, Japan) by a blind and calibrated examiner. Histomorphometric evaluation was carried out in six histological fields in the soft and mineralized tissue in the central region of the interface of bone graft and recipient site (calvarial bone).

Regarding to bone tissue, histomorphometric parameters were mature bone (characterized by bone tissue organized in lamellae, with fewer osteocytes), immature bone (characterized by less tissue organization and greater number of osteocytes), empty osteocyte lacunae, lacunae filled by osteocyte, bone marrow, osteoblasts, osteoclasts, blood vessels (Havers system), white spaces (possibly related to areas of resorption), necrosis area, and soft tissue between the bone-grafted area. Regarding

soft tissue, collagen fibers, fibroblasts, blood vessels, multinucleated giant cells, inflammatory cells osteoblasts, osteoclasts, adipocytes, white spaces (possibly occupied by interstitial and fluid edema) and necrosis area were evaluated. These histological evaluations were performed by software Image J 1.50 (ImageJ, National Institutes of Health, Bethesda, Maryland, USA) using the plug-in "grid" and "point tool".

Statistical analysis

Initially, histomorphometric data (implants and bone-grafted areas) were submitted to the Shapiro-Wilk normality test. In the sequence, a two-way ANOVA was applied to evaluate histological data from all groups, followed by Tukey's test. MicroCT results were also evaluated by two-way ANOVA. The significance level was always set at 0.05 and all calculations were performed using GraphPad program Prism 8.0 (GraphPad Software Inc, EUA).

RESULTS

MicroCT analysis – implant area

All evaluated MicroCT parameters (BIC, BAFO, BS/BV, Tb.Th, Tb.N, Tb.Sp) of the implant area did not show statistically significant difference between adhesive and screw groups, time (30 and 45 days) and double interaction group*time ($p > 0.05$) (Table 1).

Histomorphometric analysis – implant area

No significant differences were detected between adhesive and screws groups regarding to BIC results (Figure 2A). Moreover, differences were not observed between different periods in groups adhesive and screw. In the same way, no differences were observed concerning to BABT findings, as illustrated in Figure 2B.

Table 1: Mean (SD) and p-values for MicroCT parameters in adhesive and screw groups at 30 and 45 days.

| *Parameter* | *Adhesive 30days* | *Adhesive 45days* | *Screw 30 days* | *Screw 45 days* | *p-value* |
|---|---|---|---|---|---|
| *BIC ($mm^3$)* | 2.06 (0.75) | 2.45 (0.67) | 2.37 (0.3) | 2.53 (0.52) | > 0.05 |
| *BAFO (%)* | 26.03 (8.33) | 26.27 (5.19) | 31.04 (3.57) | 32.89 (6.56) | > 0.05 |
| *BS/BV (1/mm)* | 95.06 (24.97) | 87.14 (18.15) | 77.38 (8.65) | 80.95 (12.67) | > 0.05 |
| *Tb.Th (mm)* | 0.04 (0.01) | 0.12 (0.18) | 0.05 (0.01) | 0.04 (0.00) | > 0.05 |
| *Tb.N (1/mm)* | 6.35 (1.11) | 6.82 (0.93) | 6.73 (0.86) | 7.46 (1.03) | > 0.05 |
| *Tb.Sp (mm)* | 0.41 (0.22) | 0.5 (0.08) | 0.44 (0.10) | 0.47 (0.09) | > 0.05 |

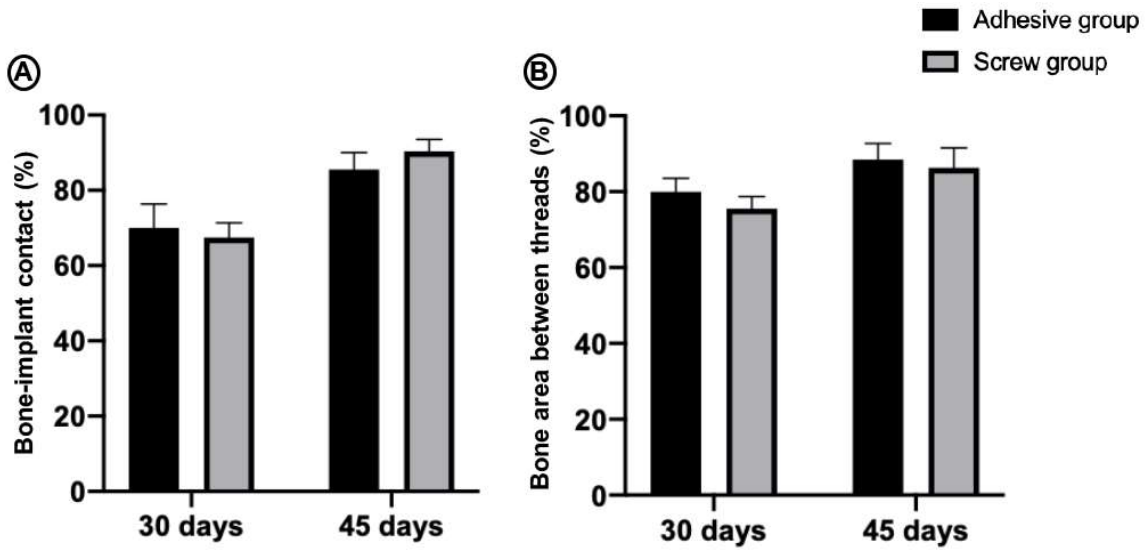


Figure 2- percentage of bone-implant contact (a) and bone area between threads (b) at 30 and 45 days in screw and adhesive groups. The results represent average values and standard deviation. Observe the absence of statistical differences between screw and adhesive groups in all experimental periods ($p<0.05$).

Histomorphometric analysis – bone-grafted areas

Histomorphometric analysis was performed in the soft and mineralized tissues of the interface between autogenous bone block and recipient site (grafted area) at 60 and 75 days. Regarding the mineralized tissues, no differences were observed in relation to the amount of mature bone between the groups in the different evaluated

periods. However, the amount of immature bone was more pronounced in the adhesive group ($p = 0.0026$) compared to screw group after 75 days (Figure 3A and 3B). No differences were observed between groups regarding the presence of empty osteocyte lacunae (Figure 3C), filled osteocyte lacunae (Figure 3D) and bone marrow (Figure 3E), osteoblasts (Figure 3F), osteoclasts (Figure 3G), blood vessels (Figure 3H), white spaces (Figure 3I), and soft tissue between mineralized areas (Figure 3J). Areas of necrosis were not detected in either group.

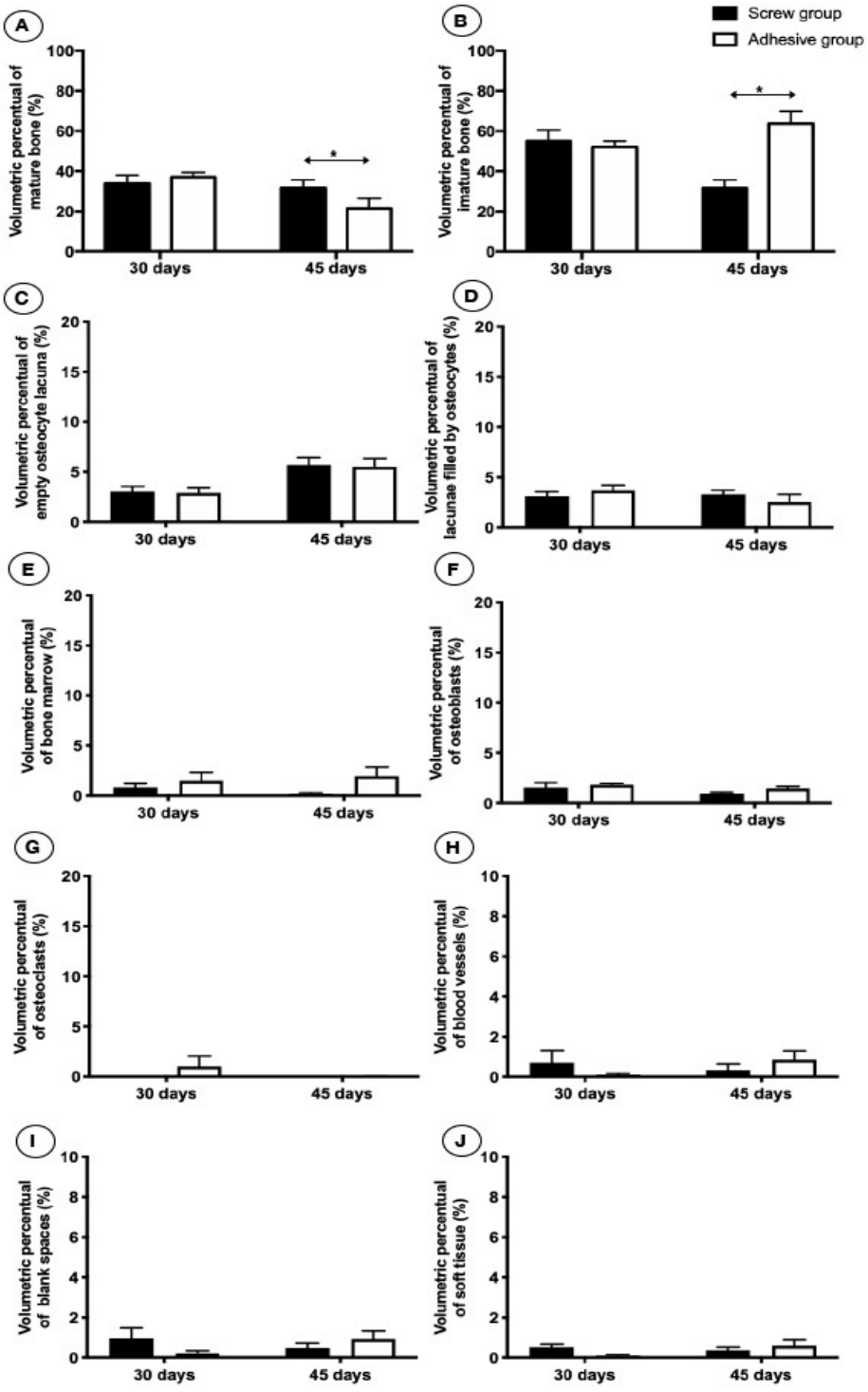


Figure 3 Volumetric density of mature bone (A), immature bone (B), empty osteocyte lacuna (C), lacunae filled by osteocytes (D), bone marrow (E), and osteoblasts (F), osteoclasts (G), blood vessels (H), white spaces (I), and soft tissue (J) in the mineralized tissues of interface between bone block and recipient site of screw and adhesive groups at 60 and 75 days of grafting procedures. The results represent average values and standard deviation. * indicate the presence of significant statically difference ($p>0.05$).

The soft tissue of this interface presented features compatible with the conditions of normality. In fact, no differences between groups were observed in respect to collagen fibers (Figure 4A), fibroblasts (Figure 4B), blood vessels (Figure 4C), inflammatory cells (Figure 4D), white spaces (Figure 4E), osteoblasts (Figure 4F), and adipocytes (Figure 4G). Multinucleated giant cells and areas of necrosis were not detected in any groups. In addition, no adhesive particles were observed in the adhesive groups in any of the experimental periods (60 and 75 days) (Figures 5 and 6).

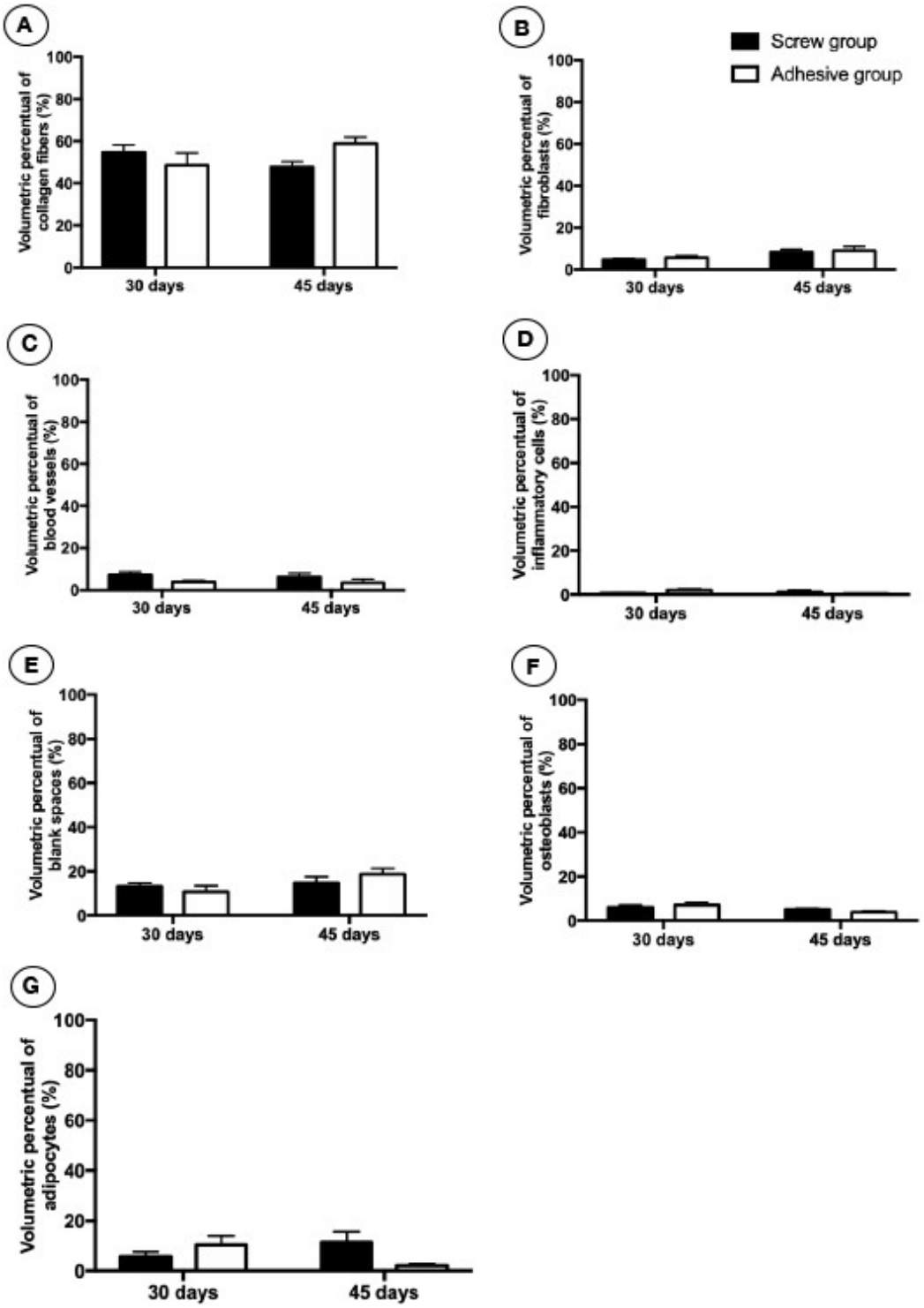


Figure 4 volumetric density of collagen fibers (a), fibroblasts (b), blood vessels (c), inflammatory cells (d), white spaces (e), osteoblasts (f), and adipocytes (g) in the soft tissues of interface between bone block and recipient site of screw and adhesive groups at 60 and 75 days of grafting procedures. The results represent average values and standard deviation. Observe the absence of statistical differences between screw and adhesive groups in all experimental periods($p<0.05$).

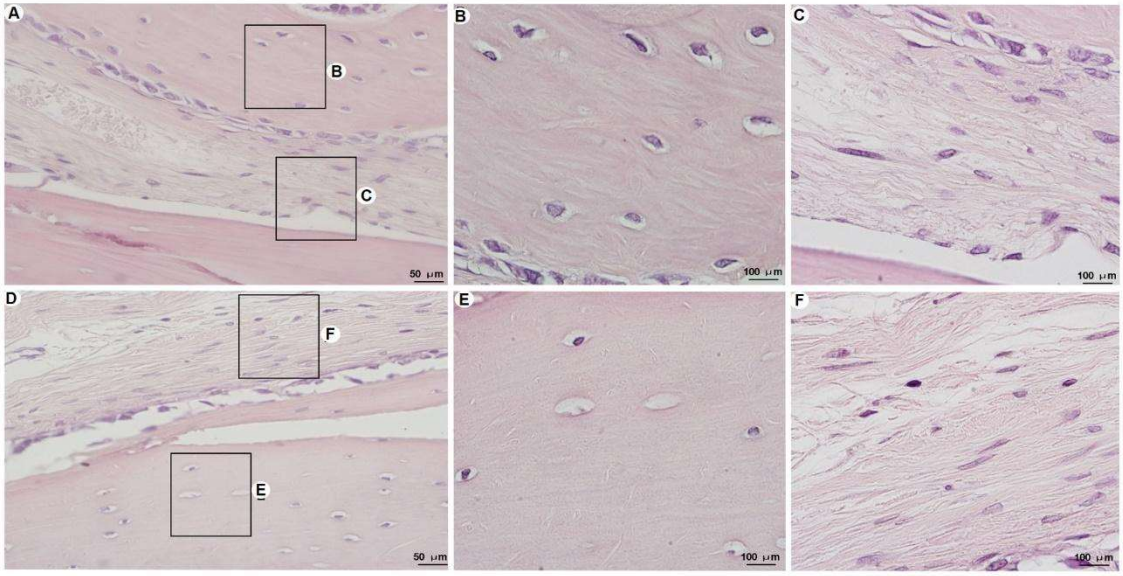


Figure 5- Histological features of screw and adhesive group after 60 days of grafting procedures. Histological sections are representative of interface region between native bone and bone graft (hematoxylin and eosin staining) of adhesive (A, B, and C) and screw (D, E, and F) groups.

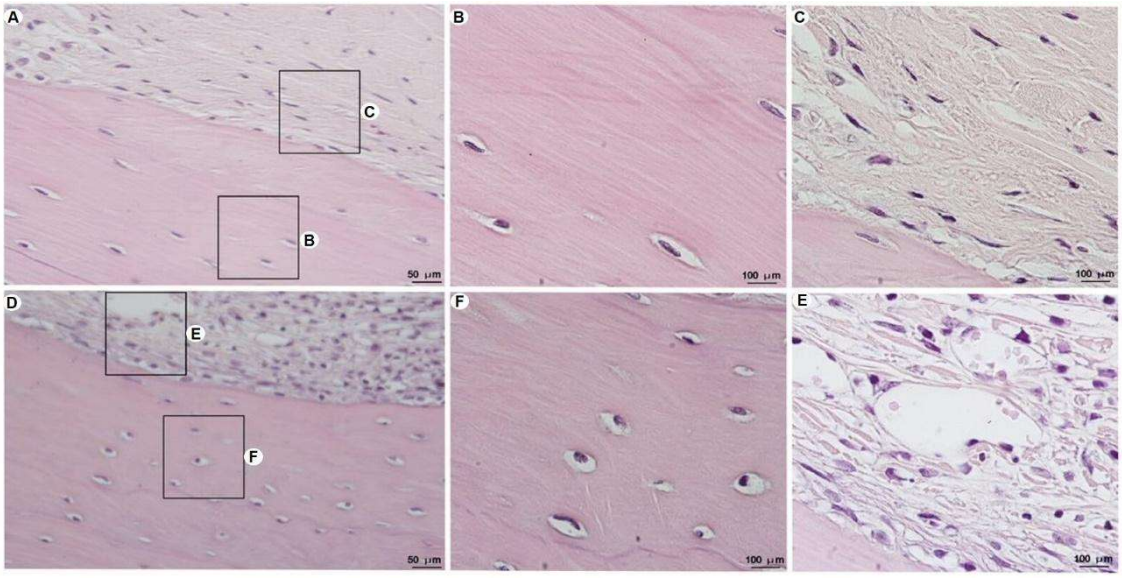


Figure 6- Histological features of screw and adhesive group after 75 days of grafting procedures. Histological sections are representative of interface region between native bone and bone graft (hematoxylin and eosin staining) of adhesive (A, B, and C) and screw (D, E, and F) groups.

## DISCUSSION

The titanium screws are described as the gold standard for the fixation of the bone graft[24,25]. In this context, the use of cyanoacrylate-based adhesive has been suggested as an alternative for fixation of bone blocks[24-28]. However, implant placement in bone-grafted area with cyanoacrylate-based adhesives not completely described. Therefore, this study was designed to assess implant osseointegration in autogenous bone blocks fixed with cyanoacrylate adhesive and screws. In addition, the histological evaluation of the grafted area was also evaluated. Our experimental design was performed in rabbits, as previously described in other studies related to grafting procedures[10,14,26,28] and osseointegration[23,29].

In the present study, no differences were detected between BIC and BABT values in screw and adhesive groups after 30 and 45 days. In the same way, no differences were observed in both groups by microtomographic findings. These results demonstrate that the grafting procedures, both fixation screws and with cyanoacrylate-based adhesive, did not influence bone deposition on the surface of the implants. In fact, some earlier studies have demonstrated high long-term implant survival rate of dental implants placed in the augmented bone, suggesting that previous grafting procedures do not influence the prognostic of rehabilitation treatment[30,31]. However, the putative influence of cyanoacrylate-based adhesives in previous grafting procedures on osseointegration has not been addressed by previous studies.

Our results also demonstrated an absence of histological differences in the soft tissues of graft interface between screw and adhesive groups. Both groups presented slight inflammatory response, characterized by discrete presence of inflammatory cells and white space (possibly occupied by inflammatory exudate), associated to absence of area of necrosis and giant multinucleated cells. In fact, other studies have described an absence of intense inflammatory reaction in soft tissue adjacent, associated to the similar cell behavior in groups fixed with ethyl-cyanoacrylate and screw of fixation[14]. According to our results, the use of cyanoacrylate-based adhesives to graft bone blocks did not affect the amount of collagen fibers, fibroblasts, blood vessels, and

osteoblasts compared to screws[32].

Controversial findings demonstrated moderate inflammatory response and delayed tissue repair after 8 weeks of grafting procedures with cyanoacrylate-based adhesives[33]. Our results concerning to mineralized tissues revealed an increase of immature bone in the adhesive group after 75 days compared to the screw group in the same experimental period. In addition, a reduction of mature bone was also detected in the adhesive group after 75 days. Adhesive particles were not observed in any experimental period.

According to the results of the present study, increased amounts of immature bone amount were detected in bone blocks fixed with cyanoacrylate, associated to more pronounced bone resorption in the screw group, which was also reported before[11]. An increased amount of immature bone in the adhesive group may suggest a delay in the maturation of bone tissue[13]. In fact, some studies have been described that perforations of bone recipient sites for screws placement can improve the grafting incorporation and bone maturation[10,13].

An important point of our experimental design is the use of self-drilling anchorage implants, which does not require bone perforations to install the implants. However, these perforations promote relevant tissue reactions, including the formation of the superficial necrosis zone. This necrotic area triggers the expression of inflammatory mediators that can stimulate bone turnover.

Taken together, our results demonstrate that not only screws but also adhesives are effective and biocompatible strategies for fixation of bone blocks. Recipient bone submitted to grafting procedures with screws and cyanoacrylate-based adhesive did not present histological differences regarding to soft tissues and increased amounts of immature bone were observed in the interface between bone block and recipient site in adhesive group after 75 days. However, these differences do not contraindicate the placement of dental implants in this bone recipient, once no differences regarding to BIC and BABT results were detected between screw and adhesive groups.

The assessment of the bone-grafted and implant areas by histology and microCT showed osseointegration. Thus, these results confirm the biocompatibility of cyanoacrylate-based adhesives and the absence of deleterious effects on bone

metabolism and osseointegration. Considering their low cost, reduction of surgical procedures, and ease of handling, the use of these adhesives can be encouraged. Moreover, an additional healing time may be required to ensure proper graft integration as well as the ability to stabilize the blocks in the presence of masticatory and occlusal forces. In this context, further studies must be carried out to improve knowledge related to biologic responses in presence of cyanoacrylate-based adhesive in grafting procedures, which may serve as a basis for the development of more effective strategies for the treatment of edentulous patients.

Acknowledgements

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REFERENCES

[1] 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; 7:219-234.

[2] Adell R, Eriksson B, Lekholm U, Brånemark PI, Jemt T. Long-term follow-up study of osseointegrated implants in the treatment of totally edentulous jaws. Int J Oral Maxillofac Implants 1990; 5:347-359.

[3] Adell R, Lekholm U, Rockler B, Brånemark PI. A 15-year study of osseointegrated implants in the treatment of the edentulous jaw. Int J Oral Surg 1981; 10:387-416.

[4] Palmquist A, Omar OM, Esposito M, Lausmaa J, Thomsen, P. Titanium oral implants: surface characteristics, interface biology and clinical outcome. J R Soc Interface 2010; 7:S515-S527.

[5] Nkenke E, Stelzle F. Clinical outcomes of sinus floor augmentation for implant placement using autogenous bone or bone substitutes: A systematic review. Clin Oral Implants Res 2009; 20:124-133.

[6] Sakkas A, Wilde F, Heufelde M, Winter K, Schramm A. Autogenous bone grafts in oral implantology: is it still a “gold standard”? A consecutive review of 279 patients with 456 clinical procedures. Int J Implant Dent 2017; 3:23.

[7] Pandit N, Pandit I. Autogenous bone grafts in periodontal practice: A literature

review. J Int Clin Dent Res Organ 2016; 8:27.

[8] Caneva M, Botticelli D, Carneiro Martins EN, Caneva M, Lang NP, Xavier SP. Healing at the interface between recipient sites and autologous block bone grafts affixed by either position or lag screw methods: a histomorphometric study in rabbits. Clin Oral Implants Res 2017; 28:1484-1491.

[9] Louis PJ. Vertical Ridge Augmentation Using Titanium Mesh. Oral Maxillofac Surg Clin North Am 2010; 22:353-368.

[10] Baş B, Özden B, Bekçioğlu B, Sanal KO, Gülbahar MY, Kabak YB. Screw fixation is superior to N-butyl-2-cyanoacrylate in onlay grafting procedure: A histomorphologic study. Int J Oral Maxillofac Surg 2012; 41:537-543.

[11] Salata La, Mariguela VC, Antunes AA, Grossi-Oliveira G, Almeida A, Taba M. Short-term evaluation of grafts fixed with either N-butyl-2-cyanocrylate or screws. Int J Clin Oral Maxillofac Surg 2014; 72:676-682.

[12] Vieira J de S, Santos FR, de Freitas JV, Baratto-Filho F, Gonzaga CC, de Araujo MR. Bond strength evaluation of cyanoacrylate-based adhesives and screws for bone fixation. Oral Maxillofac Surg 2016; 20:157–160.

[13] Oliveira Neto PJ, Cricchio G, Hawthorne AC, Okamoto R, Sennerby L, Lungren S. Tomographic, histological, and immunohistochemical evidence on the use of n-butyl-2-cyanoacrilate for onlay graft fixation in rabbits. Clin Implant Dent Relat Res 2012; 14:861-871.

[14] Saska S,Gabrielli MFR. Fixation of autogenous bone grafts with ethyl-cyanoacrylate glue or titanium screws in the calvaria of rabbits. Int J Oral Maxillofac Surg 2009; 38:180-186.

[15] de Azevedo CL, Marques MM, Bombana AC. Cytotoxic effects of cyanoacrylates used as retrograde filling materials: an in vitro analysis. Pesqui Odontol Bras 2003; 17:113-118.

[16] Harper MC, Ralston M. Isobutyl 2-cyanoacrylate as an osseous adhesive in the repair of osteochondral fractures. J Biomed Mater Res 1983; 17:167-177.

[17] Greer RO Jr. Studies concerning the hsitotoxicity of isobutyl-2-cyanoacrylate tissue adhesive when employed as an oral hemostat. Oral Surg Oral Med Oral Pathol 1975; 40:659-669.

[18] Zhang S, Guido AR, Jones RG, Curry BJ, Burke AS, Blaisdell ME. Experimental study on the hemostatic effect of cyanoacrylate intended for catheter securement. J Vasc Access 2019;20:79-86.

[19] Bao Z, Gao M, Sun Y, Nian R, Xian M. The recent progress of tissue adhesives in design strategies, adhesive mechanism and applications. Mater Biol Appl 2020;111:110796.

[20] Chen W, Lin C, Hsieh C, Tu I, Chen WYW, Hu F. Comparison of the bacteriostatic effects, corneal cytotoxicity, and the ability to seal corneal incisions among three different tissue adhesives. Cornea 2007;26:1228-34.

[21] Hida RY, Silva CB, Kusabara ILR, Mimica LMJ. Short-chain cyanoacrylates and long-chain cyanoacrylates (Dermabond) have different antimicrobial effects. BMJ Open Ophthalmol 2021 17;6:e000591.

[22] Mendes EM, Irie MS, Rabelo GD, Borges JS, Dechichi P, Diniz RS, Soares PBF. Effects of ionizing radiation on woven bone: influence on the osteocyte lacunar network, collagen maturation, and microarchitecture. Clin Oral Investig 2020;24:2763-2771.

[23] Soares PB, Moura CC, Claudino M, Carvalho VF, Rocha FS, Zanetta-Barbosa D. Influence of implant surfaces on osseointegration: a histomorphometric and implant stability study in rabbits. Braz Dent J 2015; 26:451-7.

[24] Esteves JC, Monteiro JM, Aranega AM, Betoni J, Sonoda CK. Utilization of ethyl eyanoacrylate and 2-octyl cyanoacrylate adhesives for autogenous bone graft fixation: Histomorphometric Study in Rats. J Oral Implantol 2014; 40:411-417.

[25] Noda K, Tanikawa R, Sugimura T, Kawasaki K, Kimura T, Izumi N, et al. Use of bioabsorbable plates for cranial. Neurol Med Chir 2009; 49:559-62.

[26] Santis E, Silva ER, Martins ENC, Favero R, Botticelli D, Xavier SP. Healing at the interface between autologous block bone grafts and recipient sites using n-butyl-2-cyanoacrylate adhesive as fixation: Histomorphometric study in rabbits. J Oral Implantol 2017; 43:447-456.

[27] Gonzalez E, Orta J, Quero C, Niemshik L, Galera R, Onay D, et al. Ethyl-2-Cyanoacrylate Fixation of the Cranial Bone Flap After Craniotomy. Surg Neurol 2000; 53:288-9.

[28] Hochuli-Vieira E, Engler Pinto ACB, Pereira-Filho VA, Saska S, Monnazzi MS. Adhesives based on butyl-cyanoacrylate for fixation of autologous bone graft: Pilot study in rabbits. Dent Traumatol 2017; 33:261-268.

[29] Yu YJ, Zhu WQ, Xu LN, Ming PP, Shao SY, Qiu J. Osseointegration of titanium dental implant under fluoride exposure in rabbits: Micro-CT and histomorphometry study. Clin Oral Implants Res 2019; 30:1038-1048.

[30] Tran DT, Gay IC, Diaz-Rodriguez J, Parthasarathy K, Weltman R, Friedman L.

Survival of Dental Implants Placed in Grafted and Nongrafted Bone: A Retrospective Study in a University Setting. Int J Oral Maxillofac Implants 2016; 31:310-7.

[31] Schwartz-Arad D, Ofec R, Eliyahu G, Ruban A, Sterer N. Long Term Follow-Up of Dental Implants Placed in Autologous Onlay Bone Graft. Clin Implant Dent Relat Res 2016; 18:449-61.

[32] Dadas B. Treatment of tripod fracture of zygomatic bone by N -2-butyl cyanoacrylate glue fixation, and its effects on the tissues. Eur Arch Otorhinolaryngol 2007; 264:539-544.

[33] Pelissier P, Casoli V, Le Bail B, Martin D, Baudet J. Internal use of n-butyl 2-cyanoacrylate (Indermil) for wound closure: an experimental study. Plast Reconstr Surg 2001; 108:1661-1666.

## 3.3 ANALYSIS OF THE TISSUE REPAIR OF CRITICAL DEFECTS IN THE CALOTTE OF RATS TREATED WITH PARTICULATE INORGANIC XENOGEN GRAFT AND COLLAGEN MEMBRANE

STATUS: SUBMETIDO

REVISTA: JOURNAL OF PERIODONTAL & IMPLANT SCIENCE

ABSTRACT

Purpose: Grafting procedures can be performed with different types of biomaterials and are indicated for insufficient bone availability. The aim of this study was to evaluate tissue repair of critical-size defects treated with particulate inorganic xenografts and collagen membranes in a rat calvarial model. Methods: Sixty-four rats were divided into four groups: SHAM (critical defect without grafting), BIO (critical defect filled with particulate inorganic xenograft), MEMB (critical defect filled with collagen membrane), and BIO+MEMB (critical defect filled with BIO + MEMB). The groups were subdivided according to experimental time (15 and 30 days, n=8). Histomorphometric analysis evaluated the presence of bone, biomaterial particles, collagen membrane and soft tissue, which was evaluated regarding to collagen fibers, fibroblasts, blood vessels, inflammatory cells, osteoclasts. Results: Histological analysis revealed that critical defect was predominantly filled with soft tissue with collagen fibers and fibroblasts after 15 and 30 days in the SHAM group. Within 15 days, only BIO group significantly increased bone deposition compared to SHAM ($p < 0.05$). Within 30 days, increased bone deposition and osteoclasts density was observed in BIO and BIO+MEMB compared to MEMB and SHAM ($p < 0.001$). Collagen membrane was resorbed after 30 days in MEMB and BIO+MEMB groups while the biomaterial was still present in the

bone defect in BIO and BIO+MEMB groups in the same period. Conclusions: The use of particulate inorganic xenograft increased bone deposition in critical defects in rat calvaria. However, the association of this biomaterial with collagen membrane did not result in additional benefits regarding bone deposition.
Keywords: Bone Remodeling; Biocompatible Materials; Rats.

## INTRODUCTION

Rehabilitation of edentulous ridges often includes placement of osseointegrated implants and has high success rates when adequate bone is available. However, tooth absence, pathological process and other conditions can lead to insufficient bone availability. In these situations, grafting procedures have been widely used to optimize the clinical results of prosthetic rehabilitations [1-3].

In grafting procedures for the reconstruction of atrophic ridges, several grafting biomaterials have been developed and can be used alone or combined with others [1,2,4]. Biomaterials can be classified as osteoinductive, osteoconductive and osteogenic [5-7]. Osteoinduction is characterized by the capacity of chemoattraction of undifferentiated mesenchymal cells, which will later differentiate into osteoblasts. Osteoconductive materials act as a framework for cell migration and subsequent deposition of bone tissue. Osteogenic materials are able to promote bone formation due to the presence of viable bone cells inside the graft [6-7]. Regarding to origin, biomaterials can be classified into four groups: (a) autogenous, obtained from the individual; (b) allogeneic, obtained from another individual of the same species; (c) xenogeneic, obtained from another species; and (d) alloplastic, synthetically produced [8]. Autogenous bone is widely used for its osteogenic potential and is considered gold standard in bone regeneration. However, it is usually associated with higher morbidity rates [9-10].

The use of xenogenous biomaterials has been widely used due to lower morbidity compared to autogenous grafts. These biomaterials are produced from the mineral phase of bovine bone and are similar to human bone in both surface morphology and chemical composition [10]. In fact, clinical and experimental studies performed with bovine hydroxyapatite have been demonstrated the biocompatibility of these materials in alveolar bone augmentation procedures [3,11]. In this context,

collagen membranes, obtained from xenogenous origin (porcine or bovine), are widely used associated with mineralized biomaterials. These membranes are biocompatible, promote hemostasis and chemotaxis for fibroblasts and osteoblasts. In addition, they are semi-permeable and allow nutrient perfusion, which optimizes bone defect repair. When associated with bone substitutes, studies have shown an increase in repair capacity [12]. However, this relationship remains partially elucidated. Then, the objective of this study was to evaluate tissue repair in critical bone defects with particulate inorganic xenogeneic bone associated with the use of collagen membranes after 15 and 30 days in rat calvarial model.

## MATERIAL AND METHODS

Male Wistar rats (Rattus norvegicus), with mean age of 4 months, weighing approximately 350g were used. During the experimental period, the animals were kept with food and water ad libitum, at 25°C and with 8 to 10 h of light exposure daily. Animals underwent a 12-hour fast prior to the surgical procedure. Animals were purchased from the Bioterium of State University of Ponta Grossa. All the experimental procedures were approved by the Animal Ethics committee (State University of Ponta Grossa - protocol 039/2018) and were conducted in accordance with the ARRIVE Guidelines.

The determination of the number of animals in each group was based on data analysis in the literature with similar methodology to Rothamel et al. [13]. A large effect size (Cohen's d) of 1.5 was assumed, with a statistical power of 80% and a significance level of 5% (G Power 3.1 software). Thus, a minimum sample size of 8 animals per group was determined (http://www.3rs-reduction.co.uk/html/6__power_and_sample_size.html).

### Experimental groups

Initially, the animals were divided into four experimental groups. The SHAM

group was composed of animals submitted only to the surgical to perform the critical defect, with the permanence of a blood clot due to the procedure, without any biomaterial. The MEMB group received only the collagen membrane (Jason®- Institut Straumann AG, Switzerland), while the BIO group received a particulate inorganic xenograft (Cerabone® Botiss biomaterials, Zossen, Straumann, Germany) inside the critical defect. The BIO+MEMB group received the particulate inorganic xenograft (Cerabone®) associated with the use of collagen membrane (Jason®). Each group was subdivided according to the experimental periods, which were 15 and 30 days (Table 1). Thus, each group consisted of 8 animals.

Table 1 – Experimental groups, biomaterial and periods of evaluation.

| Group | Biomaterial | Experimental | *N* |
|---|---|---|---|
| SHAM 15 | None | 15 d | *8* |
| SHAM 30 | None | 30 d | *8* |
| MEMB 15 | Collagen | 15 d | *8* |
| MEMB 30 | Collagen | 30 d | *8* |
| BIO 15 | Particulate | 15 d | *8* |
| BIO 30 | Particulate | 30 d | *8* |
| BIO/MEMB | Particulate | 15 d | *8* |
| BIO/MEMB | Particulate | 30 d | *8* |

**Surgical procedures**

Thirty minutes before the surgical procedures, the animals received morphine sulfate (3 mg/kg) (União Química, Jabaquara, SP, Brazil) intramuscularly. After 1 hour, the animals were anesthetized with 10% ketamine hydrochloride (Ceva Saúde Animal Ltda,Paulinia, SP, Brazil), at a dose of 90mg/kg associated with 2% xylazine hydrochloride (Syntec, Tambore, SP, Brazil), at a dose of 10mg/kg intraperitoneally. Trichotomy of the frontoparietal region was performed, followed by vigorous asepsis using povidone-iodine. A longitudinal mucoperiosteal incision of approximately 2

centimeters was performed, following the median sagittal suture, with a No. 15 scalpel blade. Full-thickness flaps were detached, completely exposing the cortical bone of the calvarial region. A central surgical defect was performed in the region of the parietal bones with a surgical motor and 16:1 reduction contra-angle, using a 5.0 mm diameter surgical trephine drill (Neodent, Curitiba, Paraná, Brazil), under abundant irrigation and continuous with saline solution and aspiration. To prevent overheating, only four defects were perfomed with each drill. External and internal cortical bones were removed with an Ochsenbein number 1 micro chisel, and the dura mater was kept intact with subsequent irrigation of the cavity with saline. Then, the cavity was irrigated with saline solution and filled with biomaterials, according to the experimental groups (Table 1). 33mg of particulate inorganic xenograft were inserted inside each defect and the size of membrane was 5x5mm. The biomaterials were in contact with the dura mater. Then the flap was repositioned and sutured with 3-0 nylon thread (Ethicon, Johnson & Johnson, São Paulo, SP, Brazil) (Figure 1). In the postoperative period, the animals were kept in cages (3 animals per cage) and food and water ad libitum. In the post-surgical period, the animals were monitored for physiological parameters, mainly to assess the degree of consciousness and pain symptoms. To control postoperative pain, animals received tramadol hydrochloride (União Química, Jabaquara, SP, Brazil), (7mg/kg subcutaneously, 12/12hs for 3 days).

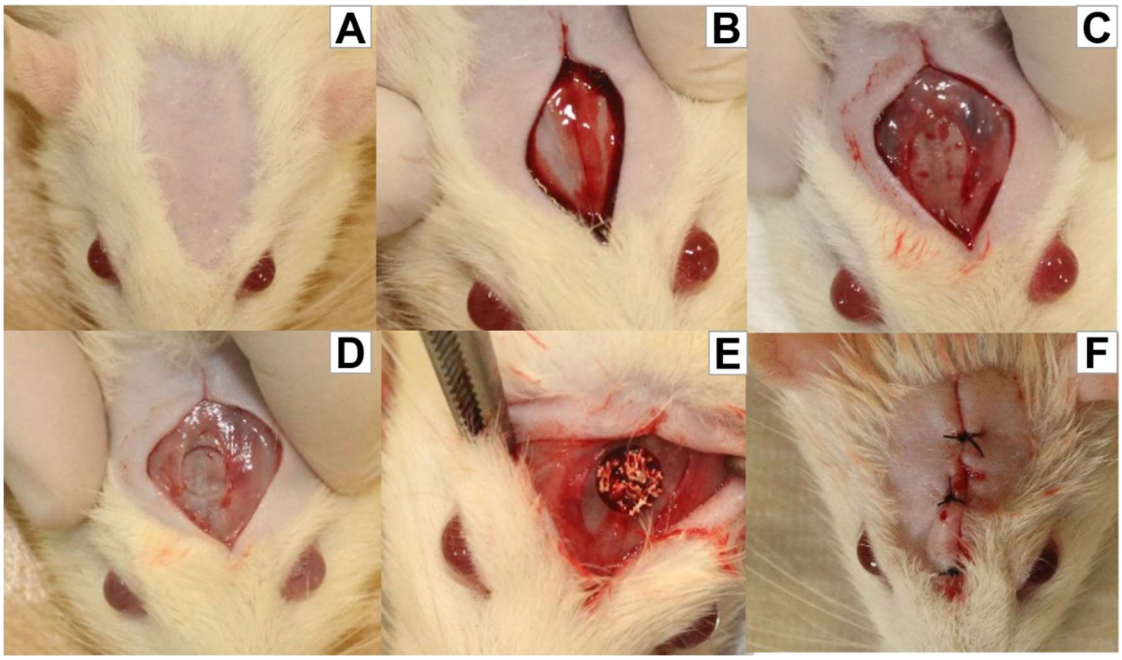


Figure 1 schematic illustration of experimental surgical procedures. Trichotomy of the cap region (a). Anteroposterior incision in the cranial vault (b). Removal of the periosteum (c). Surgical defect with a 5 mm trephine drill in the parietal bone to obtain the critical defect (d) filling the defect with the material (e) and suturing the aponeurotic fascia of the galea (f).

During the experimental follow-up period (15 and 30 days), the animals were kept at a temperature of 21°C, humidity of 65%, and light and dark cycles of 12/12 hours. Inspection and cleaning of the surgical wound was performed daily. The suture was removed after 7 days. Euthanasia was performed with an anesthetic overdose with 270 mg/Kg of 10% ketamine (Ceva Saúde Animal Ltda, Paulinia, SP, Brazil), associated with 30 mg/Kg of 2% xylazine hydrochloride (Syntec, Tambore, SP, Brazil) intraperitoneally.

After euthanasia, the defect area was subjected to trichotomy for sample collection. Next, samples were fixed in 10% buffered formalin solution for 48 hours and decalcified in 10% EDTA solution (pH 7.4) for approximately 12 weeks. Then, gradual dehydration in ethanol was carried out, followed by xylene washing and paraffin embedding. Semi-serial sections 4- to 6-µm thick with a 10 µm interval were obtained and stained with hematoxylin and eosin. In all sections, a random numerical sequence was assigned to codify the experimental periods and groups throughout the analysis and reduce bias. Images were captured with a camera coupled to a microscope (SDC-

310 Samsung, Korea), and histomorphometric evaluation was carried out using ImageJ® software (Version 1.5a Wayne Rasband, Bethesda, MA, USA) by a blinded and calibrated examiner.

### Histological and Histomorphometric Analysis

Initially, total defect area was measured, followed by immature bone area, biomaterial particles area, collagen membrane area and soft tissue area. The vitality of the mineralized tissue was assessed by the presence of osteocytes in the bone gaps. In the sequence, the soft tissue was evaluated by the presence of collagen fibers, fibroblasts, blood vessels, inflammatory cells, osteoclasts. In this evaluation, the plug-in “Grid” was used for the insertion of 80 equidistant points, which were counted using the plug-in “point tool”. These results represented volumetric density of each parameter evaluated.

### Statistical Analysis

Initially, the data were submitted to the Kolmogorov-Smirnov normality test and revealed normal distribution. Two-way ANOVA was employed to evaluate histological data of all groups, followed by one-way ANOVA and Tukey’s test to determine significant differences within the same group related to time. Significance level was set at $P<0.05$. Data was analyzed using GraphPad Prism 7.0 (Graphpad Software Inc., San Diego, CA, USA).

## RESULTS

Histological analysis revealed increased bone deposition inside the defect in the BIO30 and BIO+MEMB30 groups compared to the SHAM30 and MEMB30 groups ($P <0.001$) (Figure 2A). Although BIO+MEMB30 group showed a reduction ($P<0.001$) in the amount of biomaterial and an increase ($P <0.001$) in soft tissue compared to BIO30, there was no significant difference in bone deposition between these groups (Figure

2B and C). Notably, the collagen membrane was completely resorbed at 30 days in the MEMB and BIO+MEMB groups (Figure 2D).

In the SHAM15 and SHAM30 groups, bone deposition within the defect was extremely slight, with almost the entire defect being filled by soft tissue (Figure 3). The MEMB groups followed this same pattern (Figure 4). However, histological patterns of BIO and BIO+MEMB groups (Figure 5 and 6, respectively) showed higher bone deposition and lower percentage of soft tissue compared to SHAM and MEMB, specially at 30 days.

Regarding to soft tissue, higher counts of collagen fibers were detected in the SHAM15 and SHAM30 groups (Figure 7A). Moreover, an increase in SHAM15 and BIO15 groups was observed in the blood vessels evaluation (Figure 7B). At 30 days, an increase was also observed in the BIO group compared to the BIO+MEMB group ($P < 0.05$). As well as the collagen fibers, the SHAM15 group presented a higher fibroblast count (Figure 7C). Regarding osteoclasts, there was an increase in the BIO15, BIO+MEMB15, BIO30 and BIO+MEMB30 groups compared to the SHAM15, SHAM30, MEMB15 e MEMB30 (Figure 7D). In addition, increased inflammatory cell counts were observed in the MEMB15 group (Figure 7E). No necrotic soft tissue

orforeign body-type granulomas were observed in any of the groups evaluated.

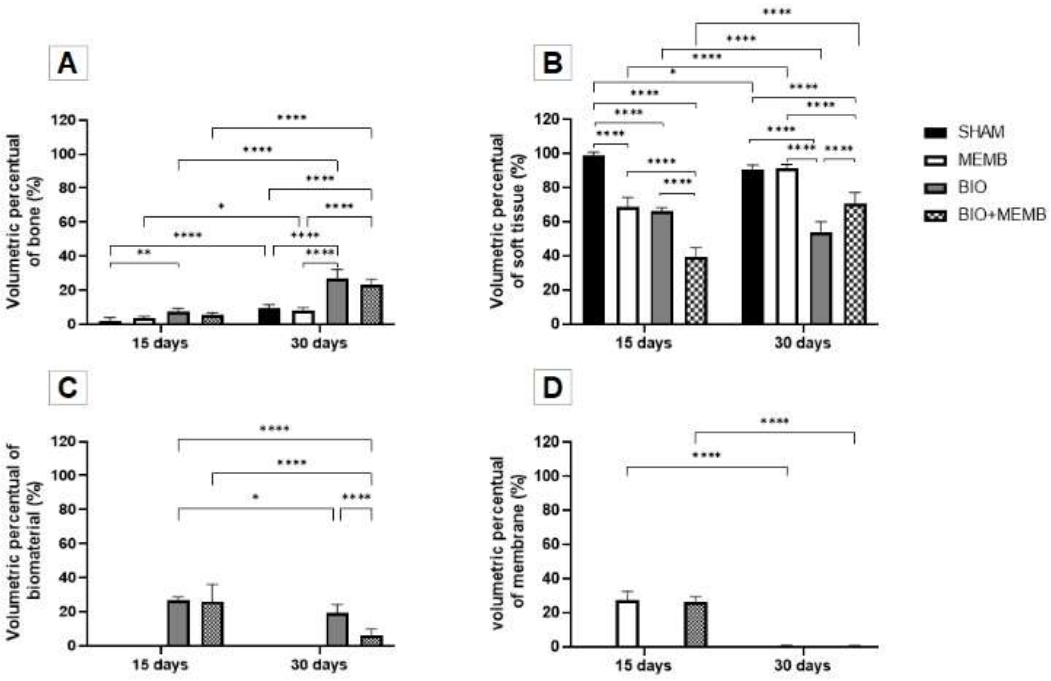


Figure 2. Quantitative evaluation of immature bone (A), soft tissue (B), biomaterial (C), and collagen membrane (D) in the calvarial critical defects. * indicates P <0.05; ** indicates P <0.01; *** indicates P <0.001; **** indicates P <0.0001.

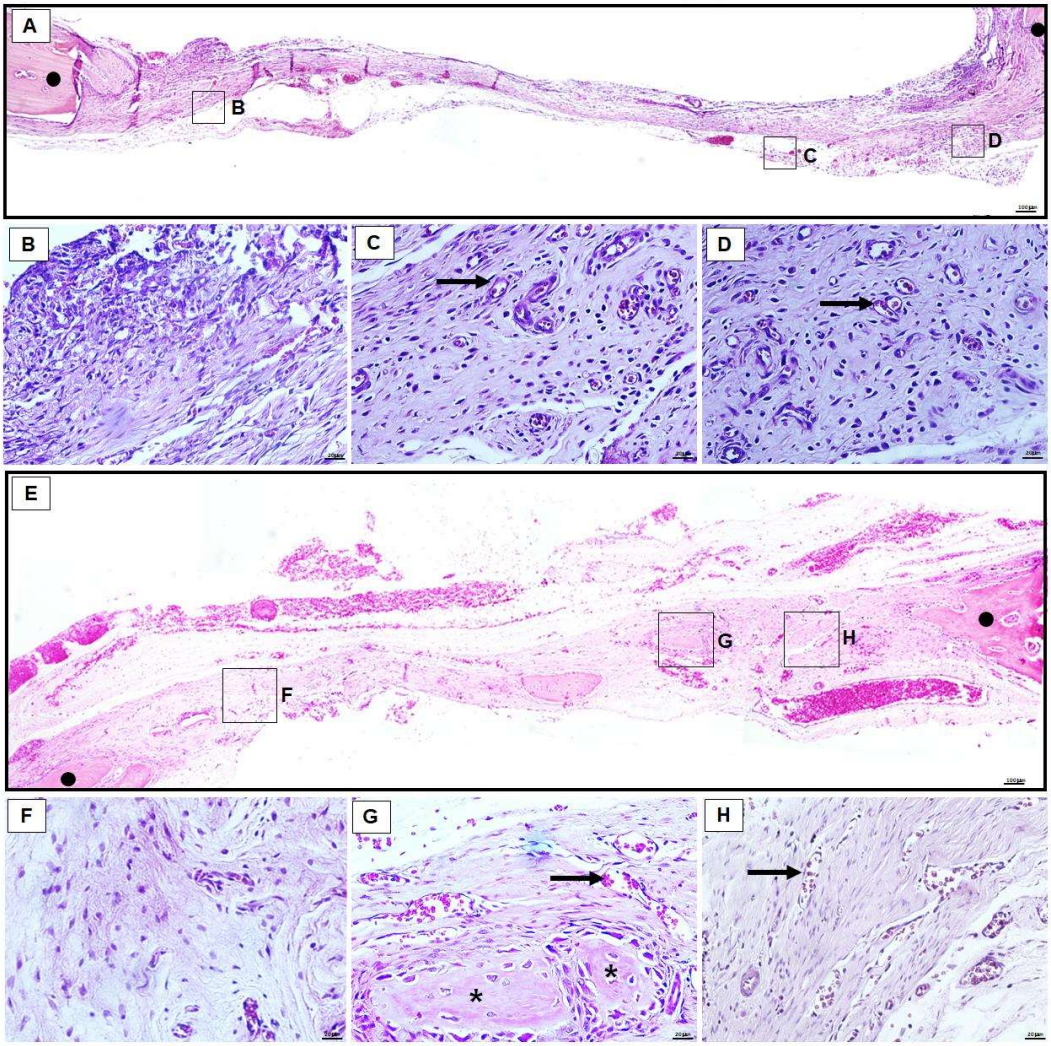


Figure 3. Bone deposition pattern of groups without the use of biomaterials (SHAM). The animals were evaluated for histological changes at 15 (A, B, C and D) and 30 (E, F, G and H) days after the grafting procedures. Black circles in A and E indicate the bone defect limits. Note bone vascularization (black arrow) and the predominance of soft tissue with minor bone deposition in 30 days (*).

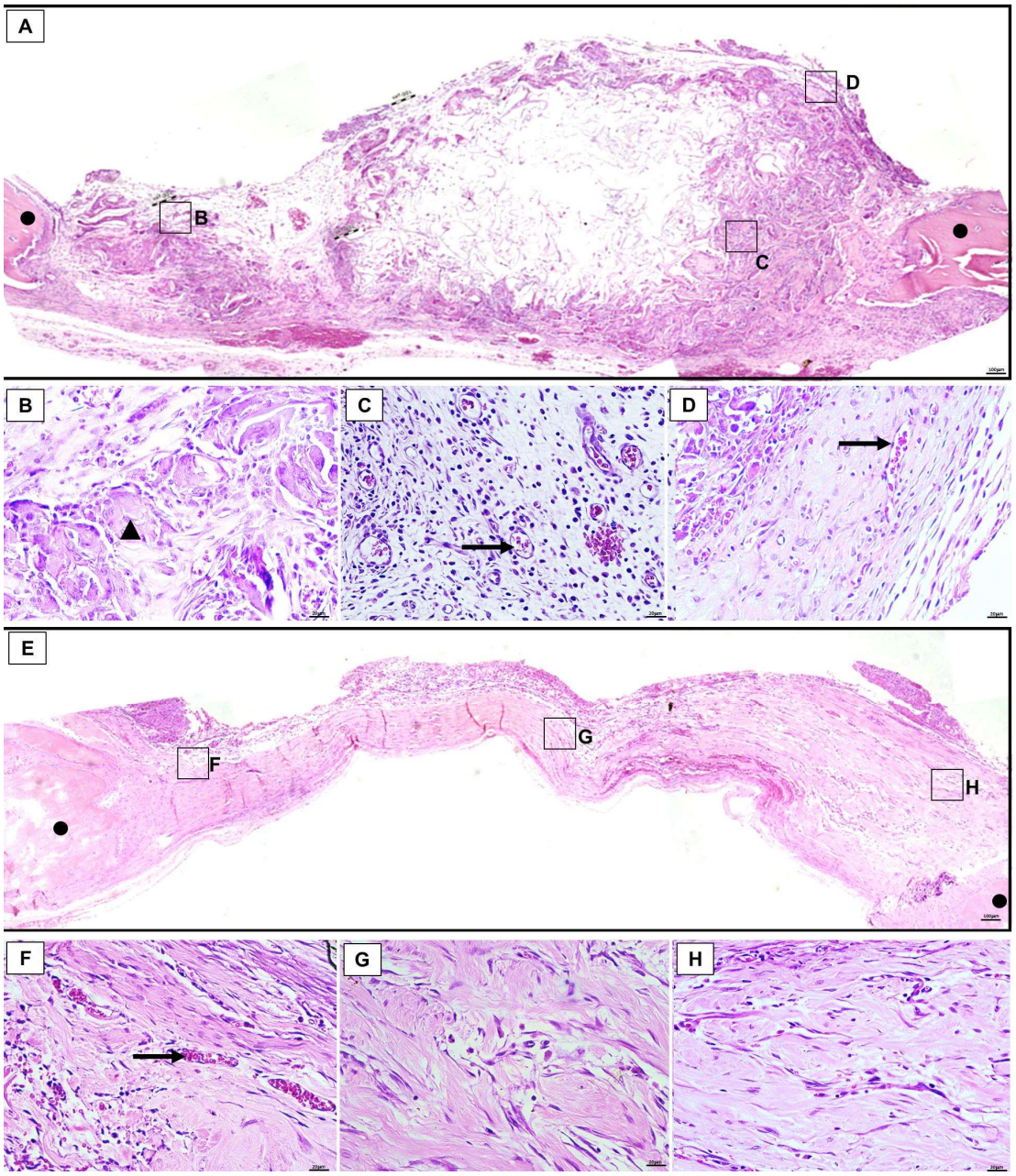


Figure 4. Bone deposition pattern of groups grafted only with collagen membrane (MEMB). Animals were evaluated for histological changes at 15 (A, B, C and D) and 30 (E, F, G and H) days after grafting procedures. Black circles in A and E indicate the bone defect limits. Note the presence of the collagen membrane (black triangle) only at 15 days. Black arrows indicates blood vessels.

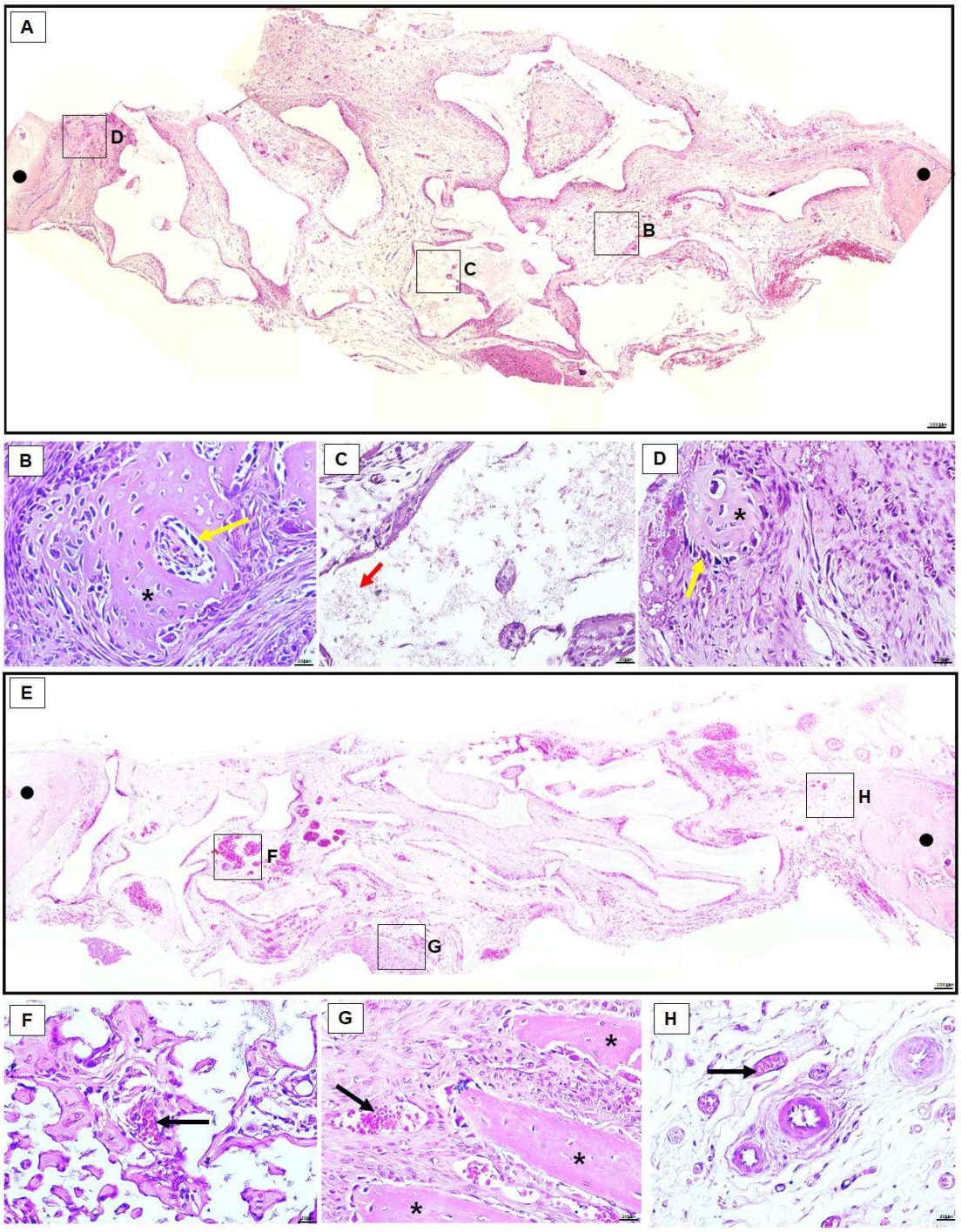


Figure 5. Bone deposition pattern of groups grafted with deproteinized bovine bone alone (BIO). Animals were evaluated for histological changes at 15 (A, B, C and D) and 30 (E, F, G and H) days after grafting procedures. Black circles in A and E indicate the bone defect limits. Note the presence of blood vessels (black arrow), biomaterial (red arrow), and the soft tissue (yellow arrow) surrounding the bone deposition (*).

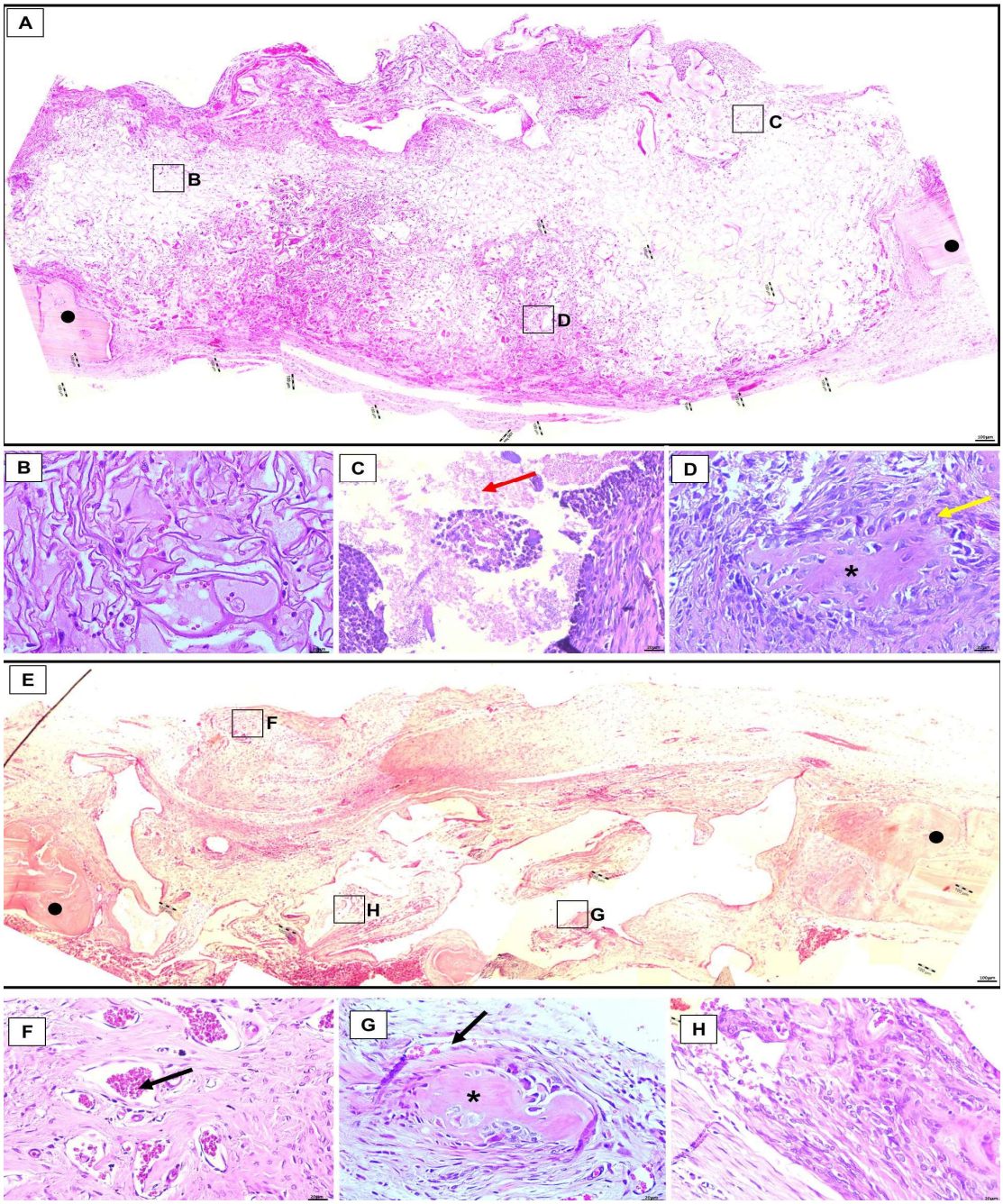


Figure 6. Bone deposition pattern of groups grafted with the association of collagen membrane and deproteinized bovine bone (BIO+MEMB). Animals were evaluated for histological changes at 15 (A, B, C and D) and 30 (E, F, G and H) days after grafting procedures. Black circles in A and E indicate the bone defect limits. Note the presence of blood vessels (black arrow), biomaterial (red arrow), bone deposition (*) and osteoblasts surrounding mineralized tissue (yellow arrow).

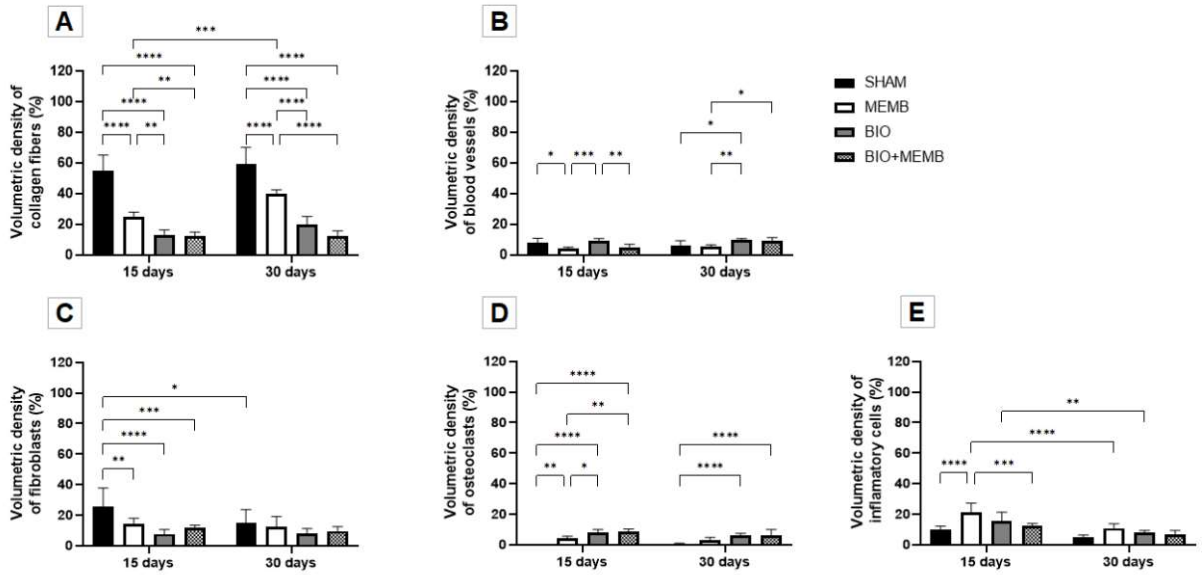


Figure 7. Quantitative evaluation of collagen fibers (A), blood vessels (B), Fibroblasts (C), Osteoclasts (D) and inflammatory cells (E) of the soft tissue in the defect. * indicates P <0.05; ** indicates P <0.01; *** indicates P <0.001; **** indicates P <0.0001.

## DISCUSSION

Several strategies to optimize bone neoformation have been developed to obtain more favorable clinical results in the rehabilitation of patients with insufficient bone availability. In this context, the association of bone substitutes associated with barriers such as resorbable membranes have been reported [14-16]. The aim of this study was to evaluate bone deposition in critical defects filled with particulate xenogenous biomaterial associated with collagen membrane, at of 15 and 30 days in a rat calvarial model.

The use of experimental models allows evaluations in pre-established periods as well as the control of factors such as defect size, environmental variations, and nutritional aspects. Among the experimental models, rats are widely described by several studies [17]. Regarding to murine model, maintenance, replicability and low cost are relevant aspects compared to animals such as dogs and monkeys [18]. Moreover, the critical defect in calvaria allows the evaluation of the interface with the

biomaterial and presents morphology and embryological origin similar to maxillary bones [19].

In this study, increased bone deposition was observed in the BIO 15, BIO 30, and BIO/MEMB 30 groups compared to the SHAM 15 and SHAM 30 groups, and demonstrated the osteoconductive profile of the biomaterials used. The SHAM 15 group showed no bone deposition within the defect. Considering that the experimental design of this study used defects of critical size, bone deposition occurred mainly from the periphery of the defect after 30 days [19-21].

Regarding the amount of biomaterial remaining in the defect, there was a significant reduction in the different experimental periods evaluated, as well as in the presence of membrane. These findings are in accordance to previous studies that evaluated biomaterials alone after 15 and 30 days [22].

When the graft was used without membrane, a greater amount of biomaterial was observed inside the defect compared to the group treated with biomaterial and collagen membrane after 30 days. This result may be associated with some factors resulting from the manufacturing process of this biomaterial, such as thermal sintering, condensed, smooth and microparticulate surface texture, in addition to the chemical structure. This induces greater production of multinucleated cells and promotes a low rate of resorption, characteristics considered favorable to xenografts [23]. This property is important, since the material becomes a scaffold, in which as the biomaterial is reabsorbed, the bone tissue is neoformed [24,25].

In fact, our results showed that the use of collagen membrane associated with particulate biomaterial increased the resorption rates of the biomaterial. However, these data were not associated with increased bone deposition. In this context, it is possible that the microenvironment created with the use of the membrane has favored osteoclastogenic activity, as observed in soft tissue evaluation. Furthermore, the defect in rat calvaria offers a microenvironment that favors the maintenance of the biomaterial in position. Thus, in bone defects with this profile, the use of collagen membranes does not seem to provide additional benefits.

Our results demonstrated that groups treated with collagen membrane showed significant resorption of this membrane. In fact, previous studies have reported a high

degradation rate of the membrane. Moreover, the biocompatibility reported in these studies was also observed in our results [26-28]. The collagen membrane used in this experimental model has collagen fibers crossed in different orientations, in order to increase the resistance to rupture and, consequently, favor guided bone regeneration [29]. In humans, complete resorption of this membrane occurs between 12 and 24 weeks. Considering that the metabolism of rodents is about 4 times faster than that of humans, the resorption of the membrane after 30 days observed in our results is in accordance with these data.

In relation to the soft tissue, a greater quantity of collagen fibers was observed in the SHAM 15 and SHAM 30 groups when compared to the other groups. Possibly, this increase is related to the absence of bone deposition areas inside the defect. These areas of bone tissue are also possibly associated with higher counts of osteoclasts in the groups with biomaterials (compared to the SHAM groups). Moreover, increased number of blood vessels and inflammatory cells was observed, possibly due to the structural characteristics of biomaterials such as composition and pore size, as well as their lower resorption rate [24,30].

Taken together, our results revealed that biomaterials are an interesting alternative to optimize bone deposition in critical rat calvarial defects and that the use of membrane was not related to increased areas of mineralized tissue. However, these data should be carefully evaluated in the context of clinical practice. Indeed, the use of experimental models is a limitation of this study compared to clinical studies. Furthermore, calvarial defects are not able to evaluate the possible influence of occlusal forces and the presence of microorganisms. Thus, further studies should be conducted to elucidate the possible mechanisms related to the factors that optimize bone metabolism in the presence of critical defects.

## CONCLUSION

The use of particulate inorganic xenograft increased bone deposition in critical defects in rat calvaria. However, the association of this biomaterial with collagen membrane did not result in additional benefits regarding bone deposition

## REFERENCES


1. Adell R, Lekholm U, Rockler B, Brånemark P. A 15-year study of osseointegrated implants in the treatment of the edentulous jaw. Int. J. Oral Surg. 1981;10(6):387–416.
2. Adell R, Eriksson B, Lekholm U, Brånemark PI, Jemt T. Long-term follow-up study of osseointegrated implants in the treatment of totally edentulous jaws. Int J Oral Maxillofac Implants. 1990;5(4):347-59.
3. Nkenke E, Stelzle F. Clinical outcomes of sinus floor augmentation for implant placement using autogenous bone or bone substitutes: a systematic review. Clin Oral Implants Res. 2009;4(20 Suppl):124-33.
4. Jemt T. Three-dimensional distortion of gold alloy castings and welded titanium frameworks. Measurements of the precision of fit between completed implant prostheses and the master casts in routine edentulous situations. J Oral Rehabil. 1995;22(8):557–564.
5. Budtz- Jörgensen E. Restoration of the partially edentulous mouth--a comparison of overdentures, removable partial dentures, fixed partial dentures and implant treatment. J Dent. 1996;24(4):237–44.
6. Greenstein G, Cavallaro J, Smith R, Tarnow D. Connecting teeth to implants: a critical review of the literature and presentation of practical guidelines. Compend Contin Educ Dent. 2009;30(7):440–53.
7. Palmquist A, Omar OM, Esposito M, Lausmaa J, Thomsen P. Titanium oral implants: surface characteristics, interface biology and clinical outcome. J R Soc Interface. 2010;7(Suppl_5):S515–27.
8. Cha HS, Kim A, Nowzari H, Chang HS, Ahn KM. Simultaneous sinus lift and implant installation: Prospective study of consecutive two hundred seventeen sinus lift and four hundred sixty-two implants. Clin Implant Dent Relat Res. 2014;16(3):337–47.
9. Cha HS, Kim JW, Hwang JH, Ahn KM. Frequency of bone graft in implant surgery. Maxillofac Plast Reconstr Surg. 2016;38(1):19.
10. Esposito M, Grusovin MG, Coulthard BP, Worthington HV. The efficacy of various bone augmentation procedures for dental implants: a Cochrane systematic review of randomized controlled clinical trials. Int J Oral Maxillofac Implants. 2005;21(5):696–710.

11. Chiapasco M. Autogenous onlay bone grafts vs alveolar distraction osteogenesis for the correction of vertically deficient edentulous ridges: a 2–4-year prospective study on humans. Clin. Oral Implants Res. 2007;18(4):432-440.

12. Clementini M, Morlupi A, Agrestini C, Barlattani A. Immediate versus delayed positioning of dental implants in guided bone regeneration or onlay graft regenerated areas: a systematic review. Int. J. Oral Maxillofac. Surg. 2013;42(5):643–650.

13. Rothamel D, Schwarz F, Fienitz T, Smeets R, Dreiseidler T, Ritter L, Happe A, Zöller J. Biocompatibility and biodegradation of a native porcine pericardium membrane: results of in vitro and in vivo examinations. Int J Oral Maxillofac Implants. 2012;27(1):146-54.

14. Roi A, Ardelean LC, Roi CI, Boia ER, Boia S, Rusu LC. Oral bone tissue engineering: Advanced biomaterials for cell adhesion, proliferation and differentiation. Mater. 2019;12(14):2296.

15. Saghiri MA, Asatourian A, Garcia-Godoy F, Sheibani N. The role of angiogenesis in implant dentistry part II: The effect of bone-grafting and barrier membrane materials on angiogenesis. Med Oral Patol Oral Cir Bucal. 2016;21(4):e526-37.

16. Iaquinta MR, Mazzoni E, Manfrini M, D'Agostino A, Trevisiol L, Nocini R, Trombelli L, Barbanti-Brodano G, Martini F, Tognon M. Innovative biomaterials for bone regrowth. Int J Mol Sci. 2019;20(3):618.

17. Mosekilde L. Assessing bone quality--animal models in preclinical osteoporosis research. Bone. 1995;17(4 Suppl):343S-352S.

18. Mosekilde L, Danielsen CC, Knudsen UB. The effect of aging and ovariectomy on the vertebral bone mass and biomechanical properties of mature rats. Bone. 1993;14(1):1-6.

19. Gomes PS, Fernandes MH. Rodent models in bone-related research: The relevance of calvarial defects in the assessment of bone regeneration strategies. Lab Anim. 2011;45(1):14-24.

20. Cooper GM, Mooney MP, Gosain AK, Campbell PG, Losee JE, Huard J. Testing the critical size in calvarial bone defects: revisiting the concept of a critical-size defect. Plast Reconstr Surg. 2010;125(6):1685-1692.

21. Spicer P, Kretlow J, Young S, Jansen JA, Kasper FK, Mikos AG. Evaluation of bone regeneration using the rat critical size calvarial defect. Nat Protoc. 2012;7(10):1918–1929.

22. Jerbić Radetić AT, Zoričić Cvek S, Tomas M, Erjavec I, Oguić M, Perić Kačarević Ž, Cvijanović Peloza O. CSBD healing in rats after application of bovine

xenogeneic biomaterial enriched with magnesium alloy. Int J Mol Sci. 2021;22(16):9089.

23. Tadic D, Epple M. A thorough physicochemical characterisation of 14 calcium phosphate-based bone substitution materials in comparison to natural bone. Biomaterials. 2004;25(6):987–994.

24. Perić Kačarević Z, Kavehei F, Houshmand A, Franke J, Smeets R, Rimashevskiy D, Wenisch S, Schnettler R, Jung O, Barbeck M. Purification processes of xenogeneic bone substitutes and their impact on tissue reactions and regeneration. Int J Artif Organs. 2018;41(11):789-800.

25. Riachi F, Naaman N, Tabarani C, Aboelsaad N, Aboushelib MN, Berberi A, Salameh Z. Influence of material properties on rate of resorption of two bone graft materials after sinus lift using radiographic assessment. Int J Dent. 2012;737262(2012):1-7.

26. Liu J, Kerns DG. Mechanisms of guided bone regeneration: A review. Open Dent J. 2014;8(6):56-65.

27. Sheikh Z, Hamdan N, Ikeda Y, Grynpas M, Ganss B, Glogauer M. Natural graft tissues and synthetic biomaterials for periodontal and alveolar bone reconstructive applications: A review. Biomater Res. 2017;21(5):9.

28. Ottenbacher N, Alkildani S, Korzinskas T, Pissarek J, Ulm C, Jung O, Sundag B, Bellmann O, Stojanovic S, Najman S, Zechner W, Barbeck M. Novel histomorphometrical approach to evaluate the integration pattern and functionality of barrier membranes. Dent J. 2021;9(11):127.

29. Ortolani E, Quadrini F, Bellisario D, Santo L, Polimeni A, Santarsiero A. Mechanical qualification of collagen membranes used in dentistry. Ann Ist Super Sanita. 2015;51(3):229-35.

30. Huber FX, Berger I, McArthur N, Huber C, Kock HP, Hillmeier J, Meeder PJ. Evaluation of a novel nanocrystalline hydroxyapatite paste and a solid hydroxyapatite ceramic for the treatment of critical size bone defects (CSD) in rabbits. J Mater Sci Mater Med. 2008;19(1):33-8.

## 4 DISCUSSÃO

Nos primórdios, os biomateriais eram definidos como biomaterial um material não vivo usado como dispositivo desenvolvido para interagir e não danificar os sistemas biológicos, mais tarde houveram alterações em suas definições, e suas funcionalidades ou seja passou a ser considerado biomaterial, um material destinado a contactar com os sistemas biológicos de forma a avaliar, tratar, aumentar ou substituir qualquer função, tecido ou órgão do organismo. Outro ponto de suma importância é que os materiais interagem com os tecidos, devem manter sua estrutura e propriedades, sem grandes alterações permanentes no meio fisiológico o qual esta exposto. Concomitantemente, devem possuir propriedades físicas e biológicas capazes de obter uma resposta positiva do organismo e não induzir reações alérgicas ou inflamatórias. Além disso, devem ainda possuir elevada osteocondutividade e bioatividade (Jebahi *et al.*, 2014. Matassi *et al.*, 2011).

Dentre os biomateriais utilizados neste trabalho, o enxerto autógeno pode ser caracterizado como uma parte ou fragmento de tecido ósseo recolhido do próprio paciente para que posteriormente possa ser colocado no local a ser induzida a neoformação óssea. (Liu *et al.*, 2019). Este tipo de enxerto segue as premissas necessárias para o processo de enxertia, pois consistem em tecido nativo transportado de uma região do corpo do paciente para outra região. Sendo desta forma considerado o “Gold standard” entre os enxertos ósseos (Brown & Laurencin, 2019). Suas vantagens incluem resistência a infecções, o que os torna excelentes no que diz respeito a capacidades de cicatrização de feridas. No entanto possuem algumas desvantagens como é o caso da morbilidade da zona dadora indicada por necrose e infecção no local da colheita, que pode causar ao paciente dor, mais elevada no local dador do que no local recetor, bem como são limitados relativamente à sua disponibilidade e quantidade (Wu *et al.*, 2019).

A incorporação do enxerto ósseo autógeno é decorrente da interação de moléculas e células do tecido enxertado e o leito receptor. A incorporação segue uma sequência específica de eventos no reparo, sendo iniciada no momento em que o bloco ósseo é envolvido por um coágulo e a reação inflamatória é iniciada. Dessa

forma, o adequado contato entre o enxerto ósseo e o leito receptor, a vascularização do local e o método de fixação são fatores extremamente relevantes na incorporação óssea (Soehardi *et al.*, 2009). A fixação rígida utilizando parafusos revolucionou os procedimentos de enxertia, neste contexto, o titânio é descrito como um metal com biocompatibilidade e propriedades mecânicas adequadas (Bae *et al.*, 2014). Novas propostas de fixação foram desenvolvidas, visando minimizar as desvantagens associadas ao uso do parafuso. Estes adesivos apresentam menor custo, facilidade de uso, rápida polimerização e ausência na produção de artefatos de imagem. Além disso, são descritos como bactericidas, hemostáticos e bioabsorvíveis. E ainda, não demandam procedimentos cirúrgicos secundários para sua remoção (Salata *et al.*, 2014).

Os enxertos xenógenos envolvem o transplante de tecido ósseo de espécies diferentes que não a espécie humana (Haugen *et al.*, 2019). Em nossos experimentos os materiais utilizados foram o (Bio-Oss®, Geistlich Pharma AG, Wolhusen, Switzerland) e o (Cerabone®, Botiss biomaterials, Zossen, Straumann, Alemanha) . Este tipo de enxerto é de origem bovina, (Orsini *et al.*, 2007, Huber *et al.*, 2006). Este tipo de enxerto ainda possui vastos desafios biológicos, no que diz respeito ao risco de resposta imunológica do tecido receptor, carência de células viáveis bem como diminuta propriedade osteoindutora (Haugen *et al.*, 2019). Estes materiais podem se apresentar de diversas formas, entre suas vantagem desse enxerto ósseo esta sua disponibilidade abundante e o custo quando comparado com outros tipos de enxertia (Moussa; Dym, 2020).

Os materiais de enxertia sintéticos são chamados na bioengenharia de aloplásticos e foram desenvolvidos com o intuito de promover uma melhoria nas propriedades de manuseio e uso especializado (Moussa; Dym, 2020). Em nosso experimento o material de escolha utilizado foi (Maxresorb® (60% HA / 40% β-TCP) (Botiss Dental GmbH, Berlin, Germany). Estes são materiais inertes, e apresentam capacidade osteocondutora em sua grande maioria (Prince *et al.*, 2019). Os materiais aloplásticos têm vindo a ganhar ênfase enquanto materiais de regeneração, pois têm a capacidade de reduzir/contrariar as complicações e desvantagens causadas pelos outros tipos de enxerto, como é o caso do risco de contaminação/transmissão de

doenças e também como o caso do risco de lesão de tecidos saudáveis, e ainda são comercializados de forma ampla o que lhes dá acesso ilimitado, bem como pelo fato de serem de fácil utilização, reduzem a morbidade do hospedeiro, diminuem o tempo cirúrgico e ainda pelo fato de estarem disponíveis no mercado sob a forma de diversos tamanhos e formas (Tumedei *et al.*, 2019). Entre muitas vantagens, destaca-se o fato de estes não possuírem antigenicidade, o que faz com que não exista risco de transmissão de doenças e ainda exista um menor risco de rejeição do enxerto pelo indivíduo receptor (Matassi *et al.*, 2011).

Outro material utilizado em nossos experimentos foram as membranas de colágeno, que tem por finalidade, quando associada ao biomaterial, realizar uma regeneração ossea guiada, esta técnica é realizada para se obter um osso com volume e qualidade adequado na área de defeito crítico com alta previsibilidade e baixo risco de complicações. Quando adicionamos a barreira mecânica, ou seja, uma membrana, espera-se que ocorra um aumento na quantidade de osso neoformado e assim maior área de regeneração tecidual, sendo maior neoformação óssea . Assim, quando estudamos o uso de diferentes membranas, o que se deseja avaliar é se o processo de ROG ocorre de maneira diferente entre elas e se a quantidade e/ou a qualidade de osso neoformado é a mesma (Costa *et al*., 2016).

As limitações referentes ao uso de modelos animais devem ser consideradas e a extrapolação destes dados para estudos clínicos deve ser feita com cautela. Neste contexto, outros estudos devem ser realizados visando otimizar as estratégias terapêuticas para reabilitação de pacientes com necessidade de procedimentos de enxertia.

## 5 CONSIDERAÇÕES FINAIS

As técnicas utilizadas para gerar a neoformação óssea, têm-se tornado cada vez mais necessárias a nível clínico com o intuito de estimular o organismo e devolver o tecido perdido ou danificado.

A grande evolução dos biomateriais, principalmente em características de osteoindução e osteocondutividade, o que tem favorecido bastante no prognóstico, tratamento e qualidade de vida dos pacientes.

O enxerto autógeno é considerado o *Gold standard*, por apresentar melhor prognóstico e aceitação pelo receptor, desta forma é considerado ideal para regeneração do tecido ósseo. Os xenoenxertos e os aloenxertos são uma alternativa válida quando comparada ao autoenxerto, e apresentam resultados satisfatórios sendo de fácil aplicação e manuseio.

O cirurgião, deve ponderar os fatores que influenciam na tomada de decisão da escolha de um biomaterial, avaliando a individualidade de cada paciente, a partir de um minucioso exame clínico e físico, levantando o estado de saúde oral e sistêmico, além da morfologia e características do local a regenerar e avaliar as vantagens e desvantagens de cada biomaterial, com o intuito de obter sucesso no processo de reparo tecidual e neoformação óssea.

# REFERÊNCIAS


ANSARI, M.. Bone tissue regeneration: biology, strategies and interface studies. **Progress In Biomaterials,** [S.L.], v. 8, n. 4, p. 223-237, 2019.

BAE, S. *et al*. Tomographic and histometric analysis of autogenous bone block and synthetic hydroxyapatite block grafts without rigid fixation on rabbit calvaria. **Journal of Periodontal & Implant Science,** v. 44, n 1, p.251-258, 2014.

BROWN, J.; LAURENCIN, C T.. Bone Tissue Engineering**. Biomaterials Science: An Introduction to Materials in Medicine.** Amsterdã: Elsevier, v.40, n1 p.1373-1388, 2019.

COSTA, N. M.F. *et al.* Osteogenic effect of tricalcium phosphate substituted by magnesium associated with Genderm® membrane in rat calvarial defect model. **Materials Science And Engineering**: C, [S.L.], v. 61, n. 1, p. 63-71, 2016.

CUNHA, M. A. G. de M. *et al.* A 15-Year Time-series Study of Tooth Extraction in Brazil**. Medicine**, [s.l.], v. 94, n. 47, p.1-7, 2015.

EMAMI, Elham *et al*. The Impact of Edentulism on Oral and General Health. **International Journal Of Dentistry**, [s.l.], v. 2013, p.1-7, 2013.

HAUGEN, H. J. *et al.* Bone grafts: which is the ideal biomaterial?. **Journal Of Clinical Periodontology**, [S.L.], v. 46, n.1, p. 92-102, jun. 2019.

HANNINK, G.; ARTS, J.J. C.. Bioresorbability, porosity and mechanical strength of bone substitutes: what is optimal for bone regeneration?. **Injury**, [S.L.], v. 42, n. 1, p. 22-25, 2011.

HUBER, F.X. *et al.* Void filling of tibia compression fracture zones using a novel resorbable nanocrystalline hydroxyapatite paste in combination with a hydroxyapatite ceramic core: first clinical results. **Arch Orthop Trauma Surg**, [S.L.], v. 126, n.1, p. 533–540, 2006.

JENSEN, S. S. *et al.* Evaluation of a novel biphasic calcium phosphate in standardized bone defects. A histologic and histomorphometric study in the mandibles of minipigs. **Clinical Oral Implants Research**, [S.L.], v. 18, n. 6, p. 752-760,2007.

JEBAHIA S. *et al.* Advances in biomaterials,new strategies, development and applications for biomedicine and engineering. **Scientific Journal of Medical Science** [S.L.] v. 3, n.2, p. 292-300, 2014

KAčAREVIć, Ž. P. *et al.* An introduction to bone tissue engineering. **The International Journal Of Artificial Organs**, [S.L.], v. 43, n. 2, p. 69-86, 2019.

KNAPIK, Derrick M. *et al.* Mechanosignaling in Bone Health, Trauma and Inflammation. **Antioxidants & Redox Signaling**, [s.l.], v. 20, n. 6, p.970-985, 2014.

LIU, Y. *et al.* Exosomes: a novel therapeutic agent for cartilage and bone tissue regeneration. **Dose-Response**, [S.L.], v. 17, n. 4, p. 1-11, 2019.

LUTZ, R. *et al.* Sinus floor augmentation with autogenous bone vs. a bovine-derived xenograft - a 5-year retrospective study. **Clinical Oral Implants Research**, [S.L.], v. 26, n. 6, p. 644-648, 2014.

MIRON, R.J.; ZHANG, Y.F.. Osteoinduction: a review of old concepts with new standards. **Journal Of Dental Research**, [S.L.], v. 91, n. 8, p. 736-744, 2012.

MUSACCHIO, E. *et al.* Tooth loss in the elderly and its association with nutritional status, socio-economic and lifestyle factors. **Acta Odontologica Scandinavica**, [s.l.], v. 65, n. 2, p.78-86,2007.

NAKATA, H. *et al.* Histological and micro-computed tomographic observations after maxillary sinus augmentation with porous hydroxyapatite alloplasts: a clinical case series. **Springerplus**, [S.L.], v. 5, n. 1, p. 1-8, 2016.

MATASSI, F. *et al.* New biomaterials for bone regeneration. **Clinical Cases in Mineral and Bone Metabolism**, , [S.L.], v. 8, n. 1, p. 21-24, 2011.

MATHEW, A. *et al.* Tissue Engineering: principles, recent trends and the future. **Nanomedicine And Tissue Engineering**, [S.L.], v.1 n.1 p. 31-82, 2016.

MOUSSA, N. T.; DYM, H.. Maxillofacial Bone Grafting Materials. **Dental Clinics Of North America,** [S.L.], v. 64, n. 2, p. 473-490, 2020.

OLIVEIRA, L. S. A. F.; OLIVEIRA, C. S.; MACHADO, A. P. L.; ROSA, F. P. Biomateriais com aplicação na regeneração óssea – método de análise e perspectivas futuras. **Revista de Ciências Médicas e Biológicas**, [S.L.], v. 9, n. 1, p. 37, 2010.

ORSINI, G. et al. Histological and ultrastructural evaluation of bone around Bio-Oss particles in sinus augmentation. **Oral Dis.**, [S.L.], v.13,n. 1 p.586–593, 2007.

PAN, Shaoxia *et al.* Does mandibular edentulous bone height affect prosthetic treatment success? **Journal Of Dentistry**, [S.L.], v. 38, n. 11, p.899-907, 2010.

PRINCE, G. E. *et al.* Yolk-porous shell biphasic bioceramic granules enhancing bone regeneration and repair beyond homogenous hybrid. **Materials Science And Engineering:** C, [S.L.], v. 100,n. 1, p. 433-444, 2019.

RODOLFO, L. M. *et al.* Substitutos Ósseos Alógenos e Xenógenos Comparados ao

Enxerto Autógeno: Reações Biológicas. **Revista Brasileira Multidisciplinar – REBRAM**, v. 20, n. 1, p. 95-105, 2017.

SALATA, L.A. *et al.* Short-Term Evaluation of Grafts Fixed With Either N-Butyl-2-Cyanocrylate or Screws. **J Oral Maxillofac Surg**, v. 72, n. 1, p.676-682, 2014.

SHEIKH, Z. *et al.* Natural graft tissues and synthetic biomaterials for periodontal and alveolar bone reconstructive applications: a review. **Biomaterials Research**, [S.L.], v. 21, n. 1, p. 1-20, 2017.

SOEHARDI, A. *et al.* The potential of the horizontal ramus of the mandible as a donor site for block and particular grafts in pre-implant surgery. **International Journal Of Oral And Maxillofacial Surgery**, v. 38, n.1, p.1173-1178, 2009.

TUMEDEI; SAVADORI; FABBRO, D. Synthetic Blocks for Bone Regeneration: a systematic review and meta-analysis. **International Journal Of Molecular Sciences**, [S.L.], v. 20, n. 17, p. 4221, 28, 2019.

WU, B. *et al.* Trends of Edentulism Among Middle-Aged and Older Asian Americans. **American Journal Of Public Health**, [S.L.], v. 103, n. 9, p.76-82, 2013.

WU, V. *et al*. Bone Tissue Regeneration in the Oral and Maxillofacial Region: a review on the application of stem cells and new strategies to improve vascularization. **Stem Cells International,** [S.L.], v. 1, n, 1. p. 1-15, 2019.

YAHAV, A. *et al*. Bone Regeneration. **Dental Clinics Of North America**, [S.L.], v. 64, n. 2, p. 453-472 2020.

# ANEXO A – PARECER DE APROVAÇÃO DA COMISSÃO DE ÉTICA NO USO DE ANIMAL

UNIVERSIDADE POSITIVO

**COMITÊ DE ÉTICA EM PESQUISA**
Rua Profº Pedro Viriato Parigot de Souza, 5.300
Fone: (41) 3317-3260 ou 3317-3290
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ceua@up.com.br

**PROTOCOLO CEUA 309**

## PARECER CONSUBSTANCIADO DE PROJETO DE PESQUISA ANALISADO PELO COMISSÃO DE ÉTICA EM PESQUISA NO USO DE ANIMAIS (CEUA - UP)

na mandíbula.
Uma incisão anteroposterior reta ao longo da região entre o dentes incisivos e pré-molares, onde será confeccionada uma incisão oblíqua posterior. Será descolado o retalho mucoperiosteal, preservando-se o feixe mentual através da localização do forame mentual. Será realizada a exodontia do dente segundo e terceiro pré-molares. Este procedimento será realizado para simular a perda dentária e necessidade clínica de enxerto ósseo. Além de evitar a perfuração do parafuso de titânio em algum dente.
Os blocos ósseos serão preparados criando-se rugosidades na superfície que ficará em contato com a mandíbula. A cortical externa da mandíbula receberá 6 perfurações monocorticais sob irrigação abundante de soro fisiológico, padronizadas pelo guia cirúrgico (o mesmo utilizado para remoção do bloco ósseo).
Durante a recuperação pós-anestésica os animais receberão 50mL de soro glicosado. Receberão o anti-inflamatório cetoprofeno injetável por via IM na dose de 1mg/kg e o antibiótico Tetraciclina injetável 25mg/kg de peso por via IM em 3 doses, a primeira dose 3 dias antes, a segunda dose trans-cirúrgica e a terceira 7 dias após a cirurgia.
No período pós-operatório, os animais serão armazenados em baias e alimentados com ração e água à vontade. Para a analgesia, será utilizada Cetoprofeno IM na mesma dose utilizada na cirurgia, durante 3 dias. A sutura será removida em 7 dias. Os animais serão clinicamente avaliados: será realizada a inspeção da ferida cirúrgica diariamente. Serão observadas as condições de higidez e saúde do animal durante as avaliações clínicas.
Cinco animais de cada grupo terão as eutanásias realizadas aos 15, 30 e 45 dias pós-operatórios. E quatro animais de cada grupo aos 45 dias receberão a instalação de implantes.
INSTALAÇÃO DOS IMPLANTES
Após 45 dias da realização dos enxertos ósseos 4 animais de cada grupo serão submetidos à instalação de implantes.
A anestesia será a mesma descrita acima.
Serão instalados implantes de 3.3 de diâmetro e 9mm Hexágono externo Titamax Ti (Neodent® - Curitiba, PR, Brasil) na região do enxerto ósseo..
Estes animais submetidos à instalação dos implantes serão eutanasiados após 60 dias.

Grupos experimentais com número de animais e tempos experimentais.

| Tempo | G1 | G2 | G3 | G4 | G5 |
|---|---|---|---|---|---|
| 15 dias | 10 | 5 | 5 | 5 | 5 |
| 30 dias | 10 | 5 | 5 | 5 | 5 |
| 45 dias | 18 | 9 | 9 | 9 | 9 |

**COMENTÁRIOS DO RELATOR**

Em outubro de 2015 foi protocolado um adendo pedindo a substituição de animais machos por fêmeas

**PARECER**

Recomenda a Aprovação

APROVADO
29/10/15

UNIVERSIDADE POSITIVO

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**PROTOCOLO CEUA 309**

PARECER CONSUBSTANCIADO DE PROJETO DE PESQUISA ANALISADO PELO COMISSÃO DE ÉTICA EM PESQUISA NO USO DE ANIMAIS (CEUA - UP)

UEPG
UNIVERSIDADE ESTADUAL DE PONTA GROSSA

**PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO**
**COMISSÃO DE ÉTICA DO USO DE ANIMAL**

CARTA DE APROVAÇÃO

**Processo CEUA** – 039/2018

**Protocolo UEPG** – 13374/2018

**Título** – Projeto de pesquisa "Análise do reparo recidual de defeitos críticos em calota de ratos tratados com enxerto xenógeno inorgânico particulado e membrana de colágeno".

**Interessados**: Leomar Emanuel Almeida Mecca/Prof. Gilson Cesar Nobre Franco-PPGO – gilsoncnf@gmail.com leoo.mecca@gmail.com

**Data de Entrada** – 30/08/2018

Prezado Leomar

A comissão de Ética no Uso de animais da Universidade Estadual de Ponta Grossa (CEUA-UEPG) certifica que os procedimentos utilizando animais no projeto de pesquisa acima especificado estão de acordo com a Diretriz Brasileira para o Cuidado e a Utilização de Animais para fins Científicos e Didáticos (DBCA), estabelecidas pelo Conselho Nacional para fins de Experimentação Animal (CONCEA) e com as normas internacionais para a experimentação animal. Dessa forma, fica autorizada a utilização de 126 (cento e vinte e seis) ratos wistar – *Rattus norvegicus* - para a execução desse projeto.

Ponta Grossa, 26 de outubro de 2018.

UNIVERSIDADE ESTADUAL DE PONTA GROSSA
Pró-Reitoria de Pesquisa e Pós-Graduação
CEUA - Comissão de Estudos e Pesquisas em Animais
Dra. Dionízia Xavier Scomparin
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