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Original Article

ASU effect on bone repair in defects grafted with bone substitute

Efeito do ASU no reparo ósseo de defeitos enxertados com substituto ósseo

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Resumo

Introdução: Área enxertadas representam uma região de qualidade biológica inferior a áreas de osso nativo. Produtos bioativos podem melhorar a qualidade óssea em áreas enxertadas. **Objetivo:** Avaliar o efeito do extrato de óleo insaponificável de abacate e soja (ASU) no reparo de defeitos críticos de calvaria (CSDs) preenchidos com substituto ósseo osteocondutor. **Material e método:** Um defeito com 0,5 mm de diâmetro foi feito em cada um dos 84 ratos. Esses defeitos foram preenchidos com coágulo (COA), osso bovino desproteinizado (DBB) ou fosfato tricálcico/hidroxiapatita (HA/TCP). ASU (0,6 g/kg) ou solução salina (CTR) foi administrada diariamente por gavagem desde 15 dias antes da cirurgia até a eutanásia dos animais (15 ou 60 dias após a cirurgia) totalizando 7 animais por período/grupo. A composição dos tecidos que preencheram os DSCs foi analisada por avaliação histomorfométrica, enquanto que a quantidade de tecido mineralizado foi avaliada por micro-CT. **Resultado:** O grupo preenchido por COA-ASU foi significativamente maior do que no grupo COA-CTR (46,40 ± 10,41% vs. 29,00 ± 8,81% aos 15 dias e 52,14 ± 6,12% vs. 42,71 ± 5,21% aos 60 dias) (p<0,05). Não houve diferenças quanto ao preenchimento ósseo entre os grupos ASU e CTR nos CSDs enxertados com DBB e HA/TCP. Houve maior quantidade de tecidos mineralizados nos CSDs dos grupos CTR do que nos grupos ASU aos 15 dias (66,73 ± 6,70% vs. 52,25 ± 9,71% nos CSDs enxertados com DBB e 53,16 ± 10,08% vs. 37,95 ± 4,70% nos CSDs enxertados com HA/TCP) (p<0,05). **Conclusão:** ASU melhorou o reparo ósseo nos CSDs preenchidos com COA; no entanto, este efeito positivo não foi observado em CSDs enxertados com DBB ou HA/TCP.

Descritores: ASU; biomateriais; reparo ósseo; defeitos críticos em calvaria; histomorfometria; micro-CT.

Abstract

Introduction: Grafted areas represent a region of lower biological quality than areas of native bone. Bioactive products can improve bone quality in grafted areas. **Objective:** To evaluate the effect of avocado/soybean unsaponifiables (ASU) on repair of critical-size calvarial defects (CSDs) filled with osteoconductive bone substitutes **Material and method:** One CCD (0.5 mm) was made in each of 84 rats. These defects were filled either with coagulum (COA), deproteinized bovine bone (DBB), or tricalcium phosphate/hydroxyapatite (TCP/HA). ASU (0.6 g/kg) or saline solution (CTR) was administered daily by gavage from 15 days before surgery until the animals were euthanized (15 or 60 days after surgery) totaling 7 animals per period/group. The composition of the tissues that filled the CSDs were analyzed by histomorphometric evaluation, while the amount of mineralized tissue was evaluated by micro–CT. **Result:** The bone filling in COA-ASU group was significantly higher than in the COA-CTR group (46.40 ±10.41% vs. 29.00 ± 8.81% at 15 days and 52.14 ± 6.12% vs. 42.71 ± 5.21% at 60 days) (p<0.05). There were no differences regarding the bone fill between the ASU and CTR groups in DBB and HA/TCP grafted CSDs. There

were higher amount of mineralized tissues in the CSDs of the CTR groups than the ASU groups at 15 days (66.73 ± 6.70% vs. 52.25 ± 9.71% in DBB grafted CSDs and 53.16 ± 10.08% vs. 37.95 ± 4.70% in HA/TCP grafted CSDs) ($p<0.05$). **Conclusion:** ASU enhanced the bone repair in the CSDs filled with COA; however, this positive effect was not seen in DBB or HA/TCP grafted CSDs.

Descriptors: ASU; biomaterial; bone healing; critical-sized calvarial defects; histomorphometry; micro CT.

INTRODUCTION

The use of bone substitute materials has become a common, and a large range of biomaterials have been proposed for that purpose[1,2]. Autogenous bone grafts are considered as the gold standard among bone substitute materials since this is the only graft which simultaneously presents the biological properties of osteogenesis, osteoinduction, and osteoconduction[3]. However, the use of autogenous bone grafts has several limitations, such as limited availability and donor site morbidity[4]. Thus, the use of biomaterials with biologically osteoconductive properties that are not taken from the patient, such as deproteinized bovine bone (DBB) and biphasic ceramics based on β-tricalcium phosphate/hydroxyapatite (TCP/HA), has been proposed as an alternative to the use of autogenous bone grafts[5].

The lack of osteoinductive and osteogenic properties of these biomaterials is related to limitations with respect to the bone regeneration/repair that they promote[3]. Alternatives, such as mixtures of these biomaterials with autogenous bone, have been proposed to improve their biological properties[4,6,7]; however, this would defeat one of the major purposes for the use of DBB and TCP/HA, which is the elimination of the need to remove a graft from a donor area of the patient. Additionally, these biomaterials have been coated with growth factors, such as recombinant human bone morphogenic protein 2 (rhBMP2)[8] and recombinant human growth and differentiation factor-5 (rhGDF-5)[9] however, the coating of biomaterials with growth factors is a high-cost procedure[10].

Avocado/soybean unsaponifiables (ASU) are used as a medication for rheumatoid arthritis and osteoarthritis[11,12] Studies have shown that ASU modifies the structures of joint tissues damaged by the progression of arthritis[13]. This phenomenon occurs due to the stimulatory effect of the ASU on the expression of growth factors such as TGFβ1, TGFβ2, and BMP[14,15], as well as on the synthesis of proteins of the connective tissue matrix (e.g., collagen and aggrecans)[15]. Additionally, preclinical studies have been showed that the systemic administration of ASU promoted a higher degree of osseointegration of dental implants placed in the tibiae of rats associated with higher expression of the growth factors TBG β1 and BMP2[16]. In addition, the use of ASU improved the periodontal repair in healthy and in animals submitted to the rheumatic arthritis induction[17,18].

Then, the aim of this study was to evaluated the effect of ASU administration on the bone repair in critical sized calvaria defects (CSDs) in rats filled with coagulum (COA), DBB and HA/TCP. Our hypothesis is that the SU may improve the bone repair in grafted areas with different osteoconductive bone substitutes.

MATERIAL AND METHOD

Distribution of the Animals and Groups

The study was approved by the School of Dentistry of Araraquara ethics committee for animal research (FOAr-UNESP, CEUA Process #01/2012). A total of eighty-four adults (3 months of age) male rats (*Rattus norvegicus*, var. Holtzman) weighing between 300-350 g were used in this study. The rats were housed in cages at a room with controlled temperature (21±1ºC) and humidity (65-70%) and a 12-hour light-dark cycle. The animals had access to standard rat chow and water ad libitum throughout the experiment. The caregivers were blinded to the treatments protocol. This study was conducted according the ARRIVE protocol.

The animals were randomized by cages using random.org and allocated into 2 groups with 3 subgroups each, which were followed for 15 or 60 days, totaling 7 animals per subgroup/period. The groups were divided according to the drug administered to the animals. In control group (CTR) saline solution was administered to the animals daily, while in ASU group (ASU) ASU (Piascledine 300®, Expanscience Lab, France) was administered to the animals daily at a dosage of 0.6 mg/kg/day. The ASU and saline solutions were administered daily by gavage, beginning 15 days before the surgical procedures until the end of the experimental period (15 or 60 days). The subgroups were divided according to the biomaterial used to fill the CSDs: in COA the CSDs were filled with coagulum, in DBB the CSDs were filled with deproteinized bovine bone graft (Bio-Oss®, Geistlich AG, Wolhusen, Switzerland), and in TCP/HA the CSDs were filled with with a biphasic TCP/HA ceramic (Straumann® Bone Ceramic, Straumann AG, Basel, Switzerland) (Figure 1).

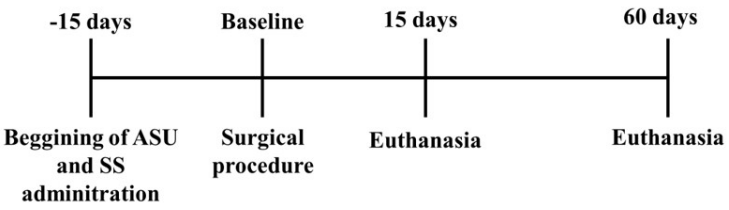

Figure 1. Flowchart of the study design of this study.

Surgical Procedure

The animals were anesthetized with a combination ketamine (0.08 ml/100 g; Rompum, Bayer S.A., São Paulo, SP, Brazil) and xylazine (0.04 ml/100 g; Rompum, Bayer S.A., São Paulo, SP, Brazil). A surgical access to the anterolateral portion of the calvaria was created through a bicoronal skin and muscle incision, with dimensions of approximately 3x2cm in the anterior and the lateral portions, respectively. Then, the scalp tissues were separated using small scissors with blunt ends and dissecting tweezers until the periosteum was exposed; it was then incised and detached to expose the bone. Then, CSDs were made in the parietal bone of the rats immediately under the apex of the coronal suture opposite the lateral incision. The CSDs were 5 mm in diameter and 1.5 mm thick. The defects were created by removal of the bone tissue by a trephine drill (3i – 3i implantes, Brasil), mounted on a low-speed hand-piece (Anthogyr – Injecta – Diadema, Brasil) under copious irrigation with sterile saline. The bone substitutes were implanted in the bone defects over the dura mater without extravasation. The soft tissues were then sutured in layers using 5.0 bioabsorbable (Vicryl, (ETHICON, J&J, São José dos Campos, Brazil) and 4.0 silk (ETHICON, J&J, São José dos Campos, Brazil) sutures. After surgery, the animals received a single intramuscular injection of a combination of penicillin and streptomycin (0.1 ml/kg) (Multibiótico Small, Vitalfarma, São Sebastião do Paraíso, MG, Brazil) for infection control and 3 days of dipyrone by gavage (0.1 ml/kg) (Dipirona Ibasa 50% - Ibasa, Porto Alegre, RS, Brazil) for pain control.

Micro CT Analysis

After the experimental periods (15/60 days) the animals from each subgroup were randomly chosen and positioned in a supine position and had their calvarias scanned by a microtomography machine (Skyscan, Aartselaar, Belgium). The images generated were then reconstructed, spatially oriented, and analyzed by specialized software (NRecon/DataViewer/CTan, Skyscan, Aartselaar, Belgium). For delimitation of regions of interest (ROIs), the images were saved in the transaxial plane as a reference and then, 40 sections that encompassed the whole defect were selected (section thickness=35 µm; 40 sections approximatelly 1.5 mm). The ROIs selected in the CTan software had a rounded shape and were similar in all animals (5x5 mm). The results were expressed as percentage of bone filling the CSD, and in the subgroups treated with the biomaterials, the analysis was performed considering a separate evaluation of the percentage of biomaterial and bone that filled the CSD. A *threshold* range between 55 and 250 in grayscale was used to evaluate the volume of mineralized tissues into de ROI (BV/TV%). A blinded, trained and calibrated examiner (GJO) performed the analyses.

Histomorphometric Analysis and Histological Description

After scanning the calvarias, the animals were euthanized through an overdose of anesthetic. Subsequently, a bicoronal incision was made in the scalp of the animal, and the entire top portion of the calvaria was removed. The samples were fixed in 4% paraformaldehyde for 48 hours and then decalcified in 7% EDTA solution for 90 days; after this period, the samples were histologically processed, embedded in paraffin, sectioned, and stained (HE).

Sections were cut from each sample beginning at the edge and continuing to the middle of the CSDs. Twenty histological slides with four sections each were prepared from each sample. For every section, five captured sections were excluded, which provided a distance of 25 µm between each section captured. The linear cross-sectional area of evaluation for each sample was 2500 μm^2 from the defect edge. For each sample, a number between 1 and 6 was drawn to determine the first slide that was stained. From the selected number, a semi-graded staining of the slides was performed, where three slides were stained and the following 3 were not stained, giving nine stained slides per sample. The third section of the first and third slides in each cluster was selected for analysis, giving six sections per sample analyzed.

The images were captured by optical microscopy (DIASTAR; Leica Reichert & Jung products, Wetzlar, Germany), with an original magnification of 25X for the histomorphometric analysis and 50X and 100X for the histological description. Because it was not possible to capture the entire CSD in a single image, it was necessary to join 2-4 images using Windows Photo Gallery software (Microsoft, Redmond, WAUSA) to include the entire defect in a single image for analysis. Determination of the diverse tissues that filled the CSDs (percentages of biomaterial, bone, and connective tissue) was performed using an image analysis program (Image J, Jandel Scientific, San Rafael, CA, USA). A blinded, trained and calibrated examiner performed the histomorphometric analyses. Furthermore, a histological description of the samples in each group was made according to the characteristics of the newly formed tissues, the presence of inflammatory cells and the relationship between the particles of biomaterials and the new bone. These analyses were performed for five animals per group in two sections close to the middle of the defect that were stained with HE and Masson's trichrome. A blinded, trained and calibrated examiner (GJO) performed the histological descriptions.

Statistical Analyses

The GraphPad Prism 5.0 (San Diego, CA, USA) software package was used to perform the statistical analyses. The sample size calculation of this study was based on a study which evaluated the effects of diverse biomaterials on the repair of CSDs in rats by applying a similar histomorphometric analysis to that used in this study[19]. It was shown that the minimum difference between the treatments regarding the average percentage of bone fill in the CSDs was 7.3% with a standard deviation of 3.75%. Therefore, when applying ANOVA, it was determined that with seven animals in each subgroup and an alpha error of 0.05, the power of the study was 80%.

The data generated by the micro-CT and histomorphometric analyses were numerical, and the Shapiro-Wilk test showed that the data were normally distributed ($p > 0.05$). ANOVA, complemented by a post-hoc Tukey test, was used for intergroup analysis for each time point. An unpaired *t*-test was used for intragroup evaluation to verify the effect of time. All tests in this study were performed with a significance level of 95% ($p < 0.05$).

RESULT

Micro CT Analysis

It was shown that within the COA subgroups, ASU groups presented a higher BV/TV% than did CTR group at 60 days ($p<0.05$). The subgroups where the CSD was grafted with the DBB and HA/TCP presented higher BV/TV% in the CTR groups at 15 days ($p<0.05$). In addition, the DBB and HA/TCP presented higher BV/TV% than the defects filled with COA ($p<0.05$), except the HA/TCP

filled defects at the ASU groups that presented the same amount or less BV/TV% than the COA at 15 and 60 days respectively. Table 1 shows the mean and standard deviation of the percentage of mineralized tissues (BV/TV%) that filled the CSD in all the groups evaluated by micro-CT.

Table 1. Mean and standard deviation of the percentage of mineralized tissues (BV/TV%) that filled the CSD in all the groups evaluated by micro-CT

Group	Biomaterial	15 days				60 days			
		BV/TV%	Tb.Th(mm)	Tb.Sp(1/mm)	Tb.N(mm)	BV/TV%	Tb.Sp(mm)	Tb.N(1/mm)	Tb.Th(mm)
CTR	COA	28.04 ± 3.90c	0.14 ± 0.02	2.69 ± 0.33	0.27 ± 0.03	34.77 ± 9.02b	0.15 ± 0.03	2.55 ± 0.21	0.26 ± 0.03
	DBB	66.73 ± 6.70*a	0.18 ± 0.03	2.41 ± 0.45	0.36 ± 0.06	57.16 ± 16.69a	0.17 ± 0.02	2.43 ± 0.29	0.34 ± 0.05
	TCP/HA	53.16 ± 10.08*b	0.13 ± 0.02	2.51 ± 0.29	0.29 ± 0.03	42.74 ± 10.56b	0.14 ± 0.03	2.48 ± 0.18	0.26 ± 0.05
ASU	COA	52.25 ± 9.71a	0.14 ± 0.03	2.72 ± 0.20	0.29 ± 0.09	51.20 ± 5.86#a	0.14 ± 0.02	2.63 ± 0.33	0.27 ± 0.05
	DBB	37.95 ± 4.70b	0.17 ± 0.01	2.61 ± 0.34	0.32 ± 0.04	40.60 ± 2.23b	0.18 ± 0.03	2.59 ± 0.41	0.34 ± 0.08
	TCP/HA	28.04 ± 3.90c	0.12 ± 0.04	2.49 ± 0.39	0.27 ± 0.05	34.77 ± 9.02b	0.13 ± 0.05	2.45 ± 0.38	0.25 ± 0.04

*Higher BV/TV% than the ASU groups; #Higher BV/TV% than the CTR groups; Different letters represent significant levels of differences between the bone substitutes within each group and period of evaluation - Two-way Anova complemented by Tukey

Histological Descriptions

At 15 days

No histological differences were observed between the CTR and ASU groups regarding DBB and HA/TCP subgroups. In the subgroups treated with bone substitutes, immature bone was found between and in contact with the particles, especially those close to the edge of the CCDs. The presence of inflammatory infiltrates was not observed, and the presence of osteoclasts was rarely observed in DBB subgroup, while osteoclasts were not observed in HA/TCP subgroup. In the center of the CSDs, an extremely large quantity of bone substitutes particles was observed in contact with disorganized connective tissue. It was also observed that when a bone substitutes was used to fill the CCD, this grafted area had similar thickness to that of the native bone. When evaluating the COA subgroup, bone formation was observed at the edges of the defect in both groups, but it was observed in the ASU group that some samples presented bone formation in the central region of the defect. The presence of a few inflammatory cells and disorganized connective tissue were also observed. Representative images from 15 days are shown in Figure 2.

At 60 days

No differences were observed between the histological patterns of the CSDs filled with DBB and HA/TCP in CTR and ASU groups. The subgroups that were filled with bone substitutes presented bone formation at the edges of the CSDs and between the particles of the bone substitutes, and in some samples, the bone was in direct contact with the particles. The particles of the bone substitutes located in the center of the defects were in contact with the connective tissue, which at this point was more organized and mature compared with the tissue at 15 days. Additionally, the particles of the bone substitutes that were close to the edges of the defects were smaller than the particles in the center of the CSDs. No inflammatory infiltrate or osteoclasts were present. It was also observed that when a bone substitutes was used to fill the CSD, this grafted area had a similar thickness to that of the native bone. In the COA subgroup, the ASU group showed bone formation in the center of the CSD that almost completely occluded the defect in some samples, but the thickness of the bone formed was thinner than that of the native bone. This pattern of bone formation was not observed in the CTR group, where bone formation was confined to the peripheral region of the CSDs. In addition, in the COA subgroups, mature connective tissue with organized collagen fibers with well-defined long axes perpendicular to the edges of the CSD was observed. Additionally, no inflammatory infiltrate was observed in these subgroups. Representative images from 60 days are shown in Figure 3.

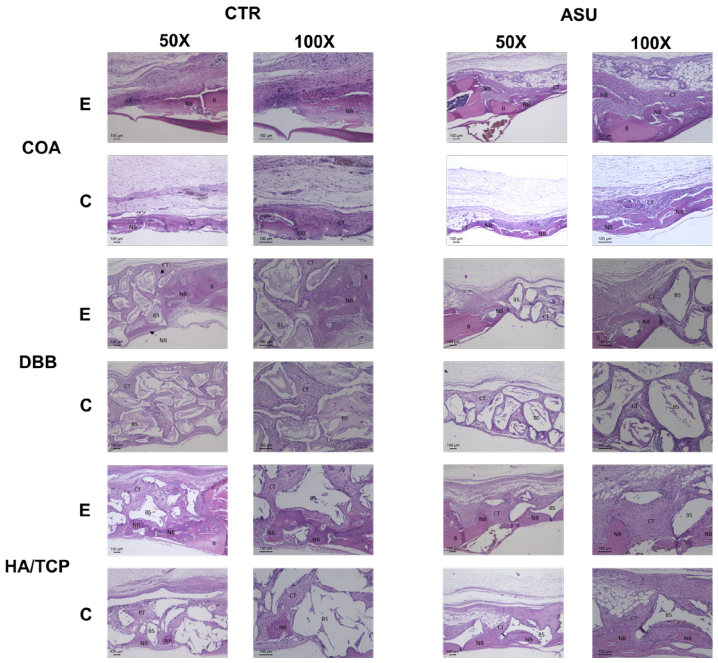

Figure 2. There were no closure of CSDs. Immature bone was found between in contact with the particles, especially those close to the edge of the CSDs. This pattern of bone formation was also observed in the COA subgroups. In the center of the CSDs it was observed an extremely large quantity of biomaterial particles was observed in contact with disorganized connective tissue. It was also observed that when the bone substitutes were used to fill the CSDs, this grafted area had similar thickness to that of the native bone. It was observed in the ASU group that some samples presented bone formation in the central region of the defect. Connective tissue (CT), Bone (B), New Bone (NB), Bone Substitute (BS), Edge of the CSDs (E), Center of the CSDs (C).

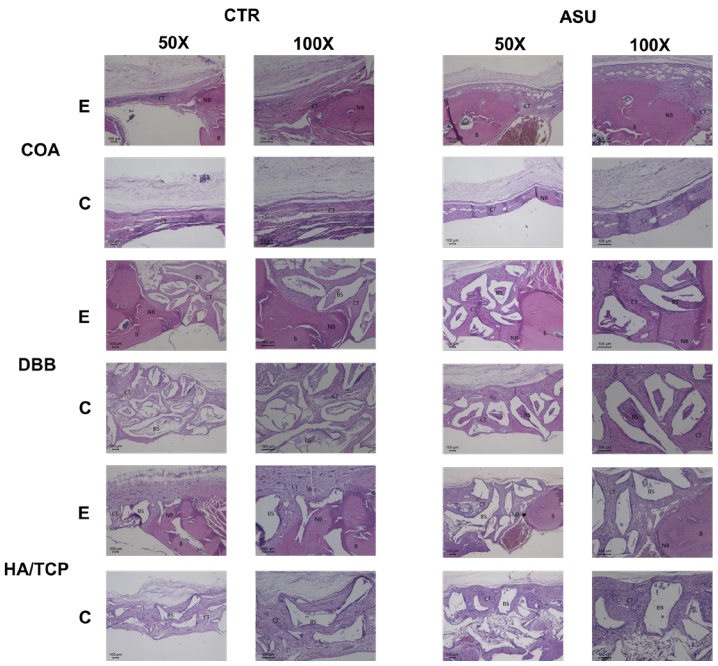

Figure 3. There were no closure of CSDs. The subgroups that were filled with DBB and HA/TCP presented bone formation at the edges of the CSDs and between the bone substitute remnant's particles. In center of the CSDs, the particles of the bone substitutes were in contact with the connective tissue, which at this point was more organized and mature compared with the tissue at 15 days. Additionally, the particles of the bone substitutes that were close to the edges of the defects were smaller than the particles in the center of the CSDs. In addition, in the COA subgroups, mature connective tissue with organized collagen fibers with well-defined long axes perpendicular to the edges of the CSD was observed. Connective tissue (CT), Bone (B), New Bone (NB), Bone Substitute (BS), Edge of the CSDs (E), Center of the CSDs (C).

Histomorphometric analysis

The COA subgroup treated with ASU presented a higher percentage of bone and a lower percentage of connective tissue than did the CTR group at the time points of 15 and 60 days ($p<0.05$). A higher percentage of bone was also verified in the COA subgroups of the ASU group compared with the DBB and HA/TCP subgroups at the time points of 15 and 60 days ($p<0.05$). In the CTR group, it was observed that the COA subgroup presented a higher percentage of connective tissue than did the DBB and HA/TCP subgroups at the time points of 15 and 60 days ($p<0.05$). Furthermore, it was observed in both groups that the percentage of biomaterial was higher in the DBB subgroups than in the HA/TCP subgroups at 60 days ($p<0.05$) (Table 2).

Table 2. Mean and standard deviation of the percentage of new bone (NB%), bone substitute remnants (BR%), and soft tissues (ST%) into de CSD in all the groups evaluated by the histomorphometric analysis

Group	Biomaterial	15 days			60 days		
		NB%	BR%	ST%	NB%	BR%	ST%
CTR	COA	29.00 ± 8.81	-	71.00 ± 8.81*a	42.71 ± 5.21*	-	58.71 ± 6.62*a
	DBB	31.43 ± 7.54	20.57 ± 7.02	48.00 ± 10.41b	39.86 ± 10.45	18.57 ± 5.65a	41.57 ± 8.84b
	TCP/HA	33.00 ± 6.48	15.86 ± 8.91	51.14 ± 7.38b	39.57 ± 8.69	11.14 ± 5.17b	49.29 ± 4.34b
ASU	COA	46.40 ±10.41#a	-	53.60 ± 10.41	52.14 ± 6.12#a	-	47.57 ± 5.99
	DBB	29.29 ± 4.53b	18.71 ± 3.25	52.00 ± 5.85	33.14 ± 4.59b	18.00 ± 3.41a	51.14 ± 3.80
	TCP/HA	33.33 ± 6.68b	15.86 ± 4.59	56.33 ± 5.68	31.71 ± 4.99b	10.33 ± 5.75b	52.43 ± 3.45

*Higher ST% than the ASU groups; #Higher NB% than the CTR groups; Different letters represent significant levels of differences between the bone substitutes within each group and period of evaluation- Two-way Anova complemented by Tuke

DISCUSSION

The results of the present study demonstrate that ASU enhanced the bone formation in the CSDs in the COA subgroups compared to the same subgroups of the CTR group, at both evaluated time points. These results confirm the findings of other studies that reported that ASU stimulated the formation of connective tissues due to up-regulation of the expression of growth factors related to bone formation, such as TGFβ1 and BMP2[13,14], and induced the synthesis of components of the connective tissue matrix[20].

However, when the bone substitutes were placed in the CSDs, differences between the ASU and CTR groups related to bone formation were not verified. Furthermore, it was shown that the COA subgroup of the ASU group presented a higher percentage of bone fill in the CSDs than did the DBB and TCP/HA subgroups. The lower amounts of bone formation in the DBB and TCP/HA subgroups may be due to the slow resorption rates of these biomaterials, which occupy the space that would eventually potentially be occupied by regenerated bone. A study that evaluated the use of bioactive glass in CSDs also detected higher bone formation in the COA group, and these authors suggested that biomaterials, which require long periods for complete resorption, will reduce new bone formation[21]. This increased bone formation in the COA group in relation to the DBB and TCP/HA groups was also observed in the CTL group at 60 days with the micro-CT analysis, but these results were not confirmed by histomorphometric analysis.

The discrepancy between the results from the micro-CT and histomorphometric analyses followed a pattern wherein the micro-CT analysis underestimated the presence of bone tissue and overestimated the presence of biomaterial. It is likely that the radiopacity of these biomaterials produces artifacts that hinder the correct identification of the bone and the biomaterial[22] . Metallic compounds that exhibit high radiopacity, such as titanium, produce artifacts that interfere with the measurement of bone tissue formation[23]. Its important to states that's the threshold definition was arbitrary and it may be also a source of variation. To the present, no methods defining a safe and precise threshold definition exist. Then, additional tests will be necessary to evaluate the different micro-CT parameters that can be modified to promote

increased agreement with the histomorphometric data associated with the repair of CSDs after the placement of different biomaterials[24].

With further regard to the histomorphometric analysis, it was verified that the COA subgroup of the CTR group presented a higher percentage of connective tissue in the CSDs than did the DBB and TCP/HA subgroups. This occurred because the presence of biomaterials maintains the space and prevents the connective tissue and soft tissue from invading the CSD[25]. However, this difference was not detected in the ASU groups. It is unlikely that accelerated bone formation in the COA subgroup treated with ASU prevented the proliferation of connective tissue into the CSD.

Regarding the biological behavior of the bone substitutes evaluated in our study, the presence of bone substitutes particles remaining inside the CSDs in the DBB and TCP/HA subgroups was verified after 60 days, which is in agreement with results from histological studies that found particles of DBB and TCP / HA remaining in grafted sites at the time of reopening for implant placement[26]. Additionally, the micro-CT and histomorphometric analyses showed that the DBB subgroup in both the CTR and ASU groups presented a higher percentage of biomaterial in the CSD than did the TCP/HA subgroups at the 60-day time point. These results confirm the results of another study which showed a higher amount of particles in sites grafted with DBB compared with sites grafted with TCP/HA[5].

Regarding the histological descriptions of the DBB and TCP/HA subgroups, the presence of bone formation between and in contact with the biomaterial particles was observed in both groups (ASU and CTR), and the size of the particles close to the edges of the CSDs was smaller than the size of the particles in the center. Furthermore, the majority of the particles in the center of the CSDs were surrounded by connective tissue. These findings confirm the osteoconductive potential of DBB and TCP/HA, which formed frameworks that guided the formation of bone around the biomaterial particles[5,27]. However, for the reduction of the volume of biomaterials to allow concomitant bone formation to occur, it is necessary for the biomaterial particles to have a close relationship with the blood supply provided by the receptor site. Two studies that compared the histology of areas grafted with DBB and TCP/HA in post-extraction sockets[28] and sinus floor augmentation[29] showed that particles of these biomaterials located far from the native bone were surrounded by connective tissue.

The small amount of bone formation promoted by the DBB and TCP / HA does not mean that it is clinically disadvantageous to use these biomaterials for the treatment of bone defects or to increase bone availability. Although the COA subgroups presented a higher percentage of bone in the CSDs, the regenerated region was thinner than the native bone that was not involved in the CSD. This finding demonstrates that the DBB and TCP/HA were more effective in maintaining the shape of the native bone, and this fact has been demonstrated in a study where DDB and TCP/HA promoted a good outcome in the preservation of bone walls[28,29].

When analyzing the data obtained in this study, some obvious limitations must be considered. ASU is a drug that alters the structure of connective tissues (e.g., bone and cartilage) at a slow rate; thus, it is not known whether the evaluation time was sufficient to identify differences in bone repair associated with DBB and TCP/HA. Factors related to dose-response effects (application of higher doses) and administration routes (local or systemic) that may also interfere with the effects of the drug were not evaluated in this study, nor was the concentration of ASU that acted directly on the CSD. Finally, the use of membranes could have interfered with the differences between the ASU and CTR groups with respect to the bone repair in the COA subgroup.

It can be concluded that ASU increased the bone repair of CSDs in the COA group compared to that ASU induced an enhancement in the percentage of bone fill in the CSDs filled with coagulum; however, this positive effect was not seen in the when DBB or TCP/HA were used. So, tour initial hypothesis that the ASU administration improve the bone repair in grafted areas with different osteoconductive bone substitutes was rejected.

AUTHORS' CONTRIBUTIONS

Lucas de Sousa Goulart Pereira: Data analysis and writing of the manuscript.
Luiz Guilherme Freitas de Paula: Acquisition and analysis of data.
Rubens Spin-Neto: Conception of the work and revision of the manuscript.
Andreas Stavropoulos: Conception of the work and revision of the manuscript.
Rosemary Adriana Chiérici Marcantonio: Conception of the work, data curation, data analysis, receipt of funding, research, methodology, project management, supervision, validation of data and experiments, design of data presentation, writing of the original manuscript, proofreading and editing.
Guilherme José Pimentel Lopes de Oliveira: Conception of the work, data curation, data analysis, receipt of funding, research, methodology, project management, supervision, validation of data and experiments, design of data presentation, writing of the original manuscript, proofreading and editing.

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CONFLICTS OF INTERESTS

The authors declare that there is no conflict of interest related to this study.

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