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Growth Dynamic of Allogeneic and Autogenous Bone Grafts in a Vertical Model

Julio Leonardo de Oliveira Lima[1], Daniel Isaac Sendyk[1], Wilson Roberto Sendyk[2], Cristiane Ibanhes Polo[1], Luciana Correa[3], Maria Cristina Zindel Deboni[1]

[1]Oral Surgery Department, School of Dentistry, USP - Universidade de São Paulo, São Paulo, SP, Brazil
[2]Oral Implantology Department, School of Dentistry, UNISA - Universidade de Santo Amaro, São Paulo, SP, Brazil
[3]Stomatology Department, School of Dentistry, USP - Universidade de São Paulo, São Paulo, SP, Brazil

Correspondence: Maria Cristina Zindel Deboni, Av. Prof. Lineu Prestes 2227, 05508-000, São Paulo, SP, Brasil. Tel: +55-11-3091-3116. e-mail: mczdebon@usp.br

Several techniques have been proposed for vertical bone regeneration, and many of them use bone autogenous and allogeneic grafts. The purpose of this study was to compare demineralised freeze-dried bone allografts (DFDBA), fresh-frozen (FF) allografts, autogenous bone grafts to find differences between volumetric and histological quantity of bone formation and vertical bone growth dynamic. A vertical tissue regeneration bone model was performed in rabbit calvarias under general anaesthesia. Four hollow cylinders of pure titanium were screwed onto external cortical bone calvarias in eight rabbits. Each one of the cylinders was randomly filled with one intervention: DFDBA, FF, autogenous bone, or left to be filled with blood clot (BC) as control. Allogeneic grafts were obtained from a ninth animal following international standardised protocols for the harvesting, processing, and cryopreservation of allografts. Autogenous graft was obtained from the host femur scraping before adapting hollow cylinders. Animals were euthanized at 13 weeks. Vertical volume was calculated after probe device measurements of the new formed tissue inside the cylinders and after titanium cylinders were removed. Histomorphometry and fluorochrome staining were used to analyse quantity and dynamic of bone formation, respectively. Results showed that DFDBA and fresh-frozen bone improved the velocity and the quantity of bone deposition in distant portions of the basal plane of grafting. Remaining material in allograft groups was more intense than in autogenous group. Both allografts can be indicated as reliable alternatives for volume gain and vertical bone augmentation.

Key Words: bone grafts, allograft, fresh-frozen bone, demineralized bone graft, osteoconduction.

Introduction

In oral rehabilitation by osseointegrated implants, the appropriate volume and contour of the alveolar ridge are crucial factors for successful functional outcomes and depends primarily on the amount of bone. The posterior region of maxilla is generally deficient in bone density and volume, and sinus lifting, grafting and vertical bone augmentation approaches are required. Several techniques have been proposed to correct defects in the alveolar bone volume and contour, such as natural bone grafts or alloplastic ceramic materials employing onlay procedures (1-4).

Current graft options include autogenous, allogeneic, and xenogeneic grafts, whose qualities vary as a function of their different biological properties. Autogenous bone is the only biomaterial that exhibits the three biological properties essential to bone reconstruction: osteogenesis, osteoinduction, and osteoconduction. Therefore, this material is considered the gold standard in grafting (1,2,5). Bone autografts without vascularisation can be categorised as block or particulate. The latter can be harvested even from intra-oral site using bone scrapers, which have the advantage of exhibiting reduced donor site morbidity but the disadvantage of a low resistance to reabsorption (4-7).

Allogeneic bone, which is transplanted from one individual to another from the same species but genetically different, represents an alternative to autografts due to advantages such as availability in tissue banks, reduced donor site morbidity, and shorter surgery time. Recently, allogeneic bone grafts have received new consideration (8) because of rigorous standards of human tissue banks in collecting and managing grafts. Fresh-frozen (FF), mineralised freeze-dried bone allograft (FDBA), and demineralised freeze-dried bone allograft (DFDBA) bone are currently available and can be employed for bone augmentation procedures and bone regeneration techniques improving quality and quantity of bone in atrophic ridges (5,7)

Although some studies have demonstrated the efficacy of autografts and allografts for the correction of alveolar defects using vertical bone augmentation techniques (3,6,9) literature is very limited in discussing the dynamic and the volume of bone neoformation, especially regarding particulate grafts.

Therefore, here we investigate comparatively demineralised freeze-dried bone allografts (DFDBA), fresh-frozen (FF), autogenous bone grafts and blood clot (as

control) to verify differences between volume, histology and vertical growth dynamic of bone formation in a vertical model in rabbit calvarias.

Material and Methods

The study was approved by the Institutional Research Ethics Committee (protocol No. 126/2009) and followed the ethical principles for animal experimentation established by the Brazilian Board of Animal Experimentation (CEUA) accordingly to ARRIVE 3Rs guidelines (10). Sample size was initially calculated at 13 animals for four experimental groups considering a significance level of 5% and a power of statistical test of 95%. But, to rationalise and reduce animal sample we considered eight animals to maintain a significance level of 5% with a reasonable power of 80% and one extra animal as a donor.

Animals

Nine New Zealand adult male rabbits weighing 3.5 to 4 kg were kept in individual cages with controlled ventilation and temperature (22±2°C), were fed a rabbit diet (Nestlé, Purina Pet Care Company, Paulínia, São Paulo- Brazil) and were given water ad libitum. Following the acclimation period, one of the animals was randomly chosen as the bone graft donor, and other eight rabbits underwent a vertical bone growth model.

Preparation of Bone Grafts

The protocols for the harvesting, processing, and cryopreservation of allografts were those used by the University Hospital Tissue Bank - University of São Paulo (BT IOT FMUSP) (11,12) that comply with the standards of the *European Association of Tissue Banks* (EATB) (13) and the *American Association of Tissue Banks* (AATB) (14).

The allografts were prepared using corticocancellous bone from the donor rabbit following euthanasia using 19.1% potassium chloride via an intravenous route under general anaesthesia. After the animal was euthanised, the distal epiphysis of the femur and proximal epiphysis of the tibia were removed, processed, and subjected to cryopreservation (-80 °C). After the microbiological analysis tested negative for bacteria and fungi, part of the allograft was fresh-frozen in -80°C in an ultra-freezer (Thermoforma®) part of bone graft was demineralised using 6% chloride acid under 4°C, after it was cryopreserved at -80°C freeze-dried, and subjected to 25 kGy of gamma radiation.

Freeze-drying was performed in two steps using an automated system including a freeze-drying chamber (Labconco®, Labconco Corporation, model 79480, Kansas City, MO, USA) and a condensing/vacuum chamber (Labconco®). During the primary freeze-drying, the largest fraction of solid-state water (ice crystals) was removed by induction of sublimation and gas migration. Motive force resulting from the pressure difference between the freeze-drying chamber and the condenser over a period of 6 hours affected the induction. At the end of the sublimation, the fraction of non-frozen water that was linked to the matrix organic components (proteins) was removed by a secondary freeze-drying. During that phase, which lasted for 4 h, the pressure in the freeze-drying chamber was raised, which was followed by a gradual increase of the temperature until positive values of up to +5 °C were achieved. At the end of the freeze-drying, the residual humidity of the allograft was 2.32%.

Autogenous bone graft was collected from the cortical of femur of each animal using a bone scraper (Conexão Sistema de Próteses, Arujá, SP, Brazil). Autogenous graft was particulate to 0.9 to 1.7 mm using a bone grinder.

The fresh-frozen fragments were reduced using an electrical bone grinder similarly to those of the autogenous, particles ranging from 0.9 to 1.7 mm. The particles of freeze-dried graft were standardised at 500 µm using a granulometry sieve.

Surgical Procedure

Aesthetic induction and maintenance were performed in all eight animals with a combination of 30–50 mg/kg ketamine (Virbac do Brazil, Roseira, SP, Brazil), 5-10 mg/kg xylazine (Agener União, São Paulo, SP, Brazil), and 5-10 mg/kg meperidine (Cristália, São Paulo, SP, Brazil) via the intramuscular route. The rabbits were given oxygen through a mask during the entire procedure.

Following anaesthetic induction and preparation of the animals, complementary infiltration anaesthesia was performed at the surgical site using 1.8 mL of 2% lidocaine with 1:100,000 epinephrine (DFL, Rio de Janeiro, RJ, Brazil). The skin incision was performed along the sagittal line and was followed by incision and periosteal dissection, thereby exposing the right and left parietal and frontal bones.

Each animal received four hollow cylinders of commercially pure titanium (Conexão Sistema de Próteses) that measured 5 mm in height and 5 mm in internal diameter and had a 2-mm side tab with two holes for fixation and stabilisation over the calvarias using titanium mini screws, as with similar devices used in previous studies (15,16)

Following the fixation of the cylinders, small perforations were made in the bone cortex to promote blood supply to the grafts. The site was abundantly cooled using irrigation with sterile 0.9% physiological saline solution.

The cylinders were randomly filled according to experimental group: demineralised freeze-dried bone allograft (DFDBA); fresh-frozen bone allograft (FF);

autogenous bone graft (AUT). The fourth cylinder was filled with blood that came from bleeding after calvarias perforations. This cylinder was left without grafting material to be used as control (BC) (Fig1A). The cylinders were then sealed with external screw caps to isolate the filling materials from the adjacent tissue and ensure graft immobility. Skin wounds were closed using a continuous suture technique with a mono-nylon 3-0 suture (Brasuture, São Paulo, SP, Brazil), which was removed 2 weeks later. Postoperatively, 5 mg/kg of 5% enrofloxacin (Baytril®, Bayer Brazil, São Paulo, SP, Brazil) and 0.2 mg/kg of 0.2% meloxicam (Maxican®, Ourofino, Cravinhos, SP, Brazil) were subcutaneously injected every day for 7 days.

Fluorochrome Labelling

Calcium-binding fluorochromes were subcutaneously administered at a dose of 20 mg/kg as follows: Alizarin (Alizarin Red ; Sigma Aldrich, St. Louis, MO, USA) at the 6th week post-surgery; calcein blue (Calcein Blue; Sigma Aldrich) at the 9th week; and green calcein (Calcein ; Sigma Aldrich) at the 12th week (16).

Sample Collection and Volume Analysis

Thirteen weeks later, the animals were euthanised under general anesthesia by an intravenous injection of 19.1% potassium chloride. The titanium cylinders were exposed, and the caps were removed. The volume of formed tissue was calculated as described previously (13,14). A millimetric Perioise probe (PDT Sensor-Probe™, Zila Dental Technologies, Batesville, AR, USA) was used to measure the distance between the edge of the cylinder and the highest point of the grown-up tissue (Fig. 1B). The probe values were recorded; the titanium cylinders were carefully removed (Fig. 1C). The samples, including the grafted material and a part of the recipient bed, were collected by an 8-mm trephine bur. Using a digital calliper, height and radius of samples were measured (Fig 1D). The volume of onlay formed tissue was calculated using the following equation: volume=$\pi r^2 h$.

Histological Preparation

Eight sample biopsies of each experimental group (n=8) were fixed in 4% phosphate-buffered formaldehyde under refrigeration for 48 h. All specimens were divided in the middle along the long axis. Half of the blocks were dehydrated in a graded series of increasing ethanol concentrations and embedded in white methylmethacrylate without being decalcified and half were decalcified in 4.13% ethylenediamine tetraacetic acid (EDTA) for 5 weeks,

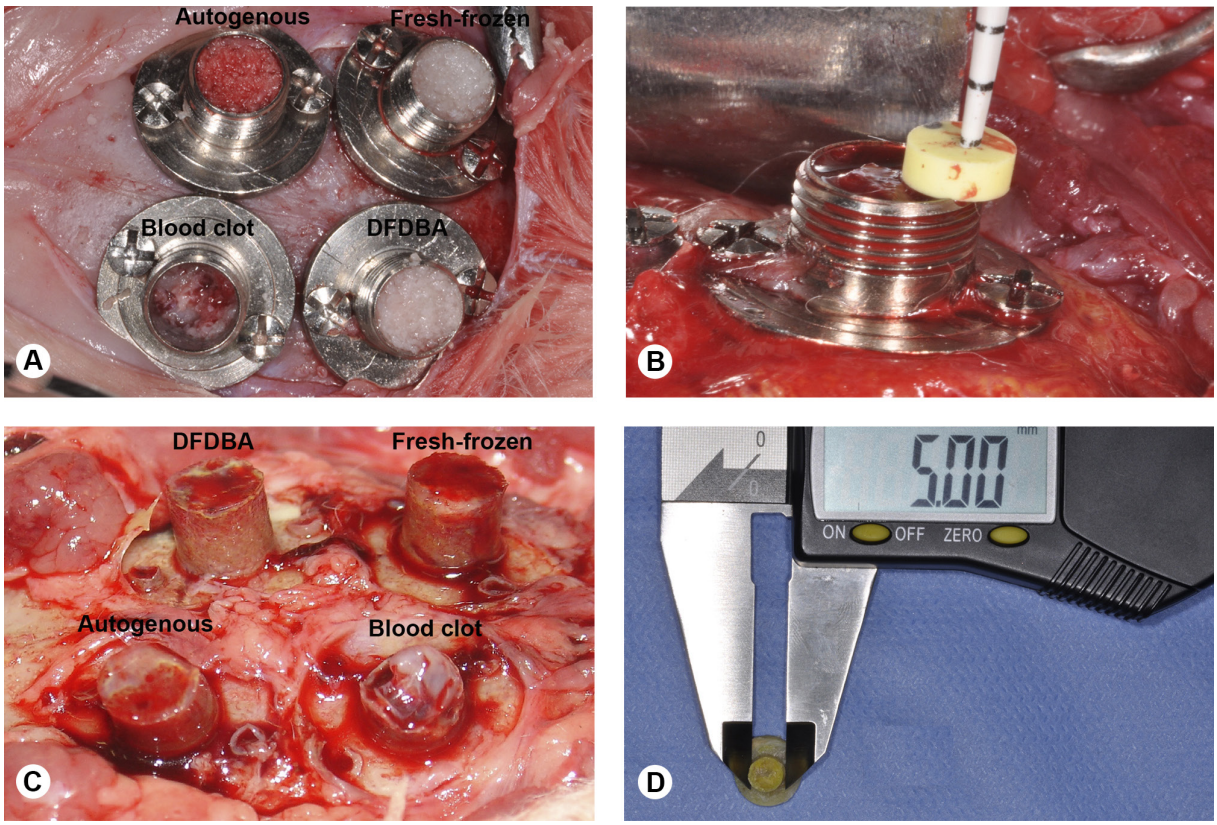

Figure 1. A: Clinical aspect of cylinders containing different bone materials and blood clot. B: Probe measuring the distance between the edge of the cylinder and the highest point of the grown-up tissue. C: Clinical aspects of the newly formed tissue after the cylinders were removed. D: Measurement of height and radius of each newly formed tissue after specimen collection.

rinsed in phosphate-buffered saline (PBS), dehydrated in a graded series of increasing ethanol concentrations, and embedded in paraffin. The specimens embedded in acrylate were prepared to section of 30 µm using a cutting and grinding system (Exakt, Nordestedt, Germany) for fluorescence analysis. Samples embedded in paraffin were sectioned along the long axis at 5µm thickness and stained in haematoxylin-eosin for histological morphology and morphometric analysis of bone formations in each group.

Histomorphometric Analysis

A conventional light microscope (Olympus® CH2, Olympus Optical Co. Ltd. Japan) was used to assess the histological sections with a final magnification of 400X. Histomorphometric analysis was adapted from a previous study (17). Briefly, four histological sections were randomly and blindly selected from each animal and group. Each histological section was divided into two halves: the apical portion of new-formed tissue distant from the recipient bed bone and the base portion near the occipital recipient bed bone. Images of the two halves were obtained at 400X magnification using microscopy (Leica DM 2500, Leica Microsystems, Wetzlar, Germany) coupled with a digital camera (Leica DFC 295, Leica Microsystems). These images were transferred to digital image processing software (ImageJ NIH - USA). Using the freehand tool, the newly formed bone in the apical and base portion of the vertical formed tissue was underlined in each specimen. The percentage area of newly formed bone for each specimen was the sum of all area percentage calculated in each portion. Similarly, remnants of allografts were underlined to be quantified as percentage remaining graft material. For the autologous group, the remaining material was quantified underlining bone fragments that showed no osteocytes stained nucleus within their lacunae. Those empty lacunae permitted to distinguish between new bone and material through haematoxylin-eosin stain.

Fluorescence Analysis

Fluorescence images were taken along the long axis of each sample block at 10X magnification (objective 2,5X) by one blinded examiner using an Axion Epifluorescence Microscope (AxioScope, Zeiss, Oberkochen, Germany). Appropriate wavelength filters were used in excitation levels: 551-573 nm for alizarin red; 358-463 nm for blue calcein, and 495-517 nm for green calcein (18). Histological sections were photographed and analysed in a coupled digital camera (Axiocam, Zeiss, Oberkochen, Germany) connected to a microcomputer. Images of each block were acquired from the apical to the base portion of the onlay-formed tissue excluding the calvarias bone region. The images were transferred to a digital image processing software (ImageJ NIH, Bethesda, MA, USA), and the fluorescence area determined by each fluorochrome individually was accessed (18). Bone formation dynamic was considered as the percentage of fluorescence-stained bone area for each fluorochrome labelling in relation to the total area of formed bone in the apical and the base portions of each block.

Data were submitted to Shapiro-Wilk normality test to determine if it was well modelled by a normal distribution. Data showed a non-parametric distribution. Kruskal-Wallis' test followed by Dunn´s test was performed with BIOSTAT 5.3 software (Fundação Maminauara, PA, Brazil). It was assumed that a p-value less than 0.05 was statistically significant.

Results

All animals showed uneventful healing of the surgical wound during the postoperative period. No losses, infections, or suture dehiscence occurred in any of the eight recipients. At the time of the sample collection, all three types of grafts (DFDBA, fresh-frozen (FF), and autogenous) and the vertical grown tissue in blood clot group (BC) were adhered to the recipient bed and showed no signs of inflammation.

Macroscopic and Volume Analysis

In all four interventions, there were onlay-formed tissue volumes inside the titanium cylinders. The median values are shown in Table 1.

Histomorphometry

Morphological features of repair showed that fresh-frozen and DFDBA groups exhibited moderate-to-intense bone neoformation. In the autogenous group, there was great amount of dense connective tissue rich with blood vessels, localised mainly in the apical portion and new bone formation was localised near the base (Fig. 2A). In the blood clot group, small amount of dense connective

Table 1. Median (range) of dimensional values for the new-formed tissue localized in the inner cylinder used for induction of bone formation.

	High (mm)	Radius (mm)	Volume (mm^3)
Autogenous	2.4 (1.3-4.5)[a,b]	2.4 (2.2-2.5)[a,b]	43.4 (19.9-88.2)[a,b]
Blood clot	0.8 (0.4-1.6)[a]	2.2 (1.7-2.4)[a]	9.9 (5.9-20.3) [a]
Fresh-frozen	4.1 (2.5-4.7)[b]	2.4 (2.3-2.5)[b]	76.3 (41.4-86.2)[b]
DFDBA	4.3 (2.2-4.6)[b]	2.4 (2.2-2.5)[a,b]	79.7 (31.4-86.6)[b]

Kruskal–Wallis' test followed by Dunn's test. Significant when $p<0.05$. [a] or [b] Different letters indicate significant differences between groups

tissue was present near the base; new bone was absent (Fig. 2B). In fresh-frozen and DFDBA groups (Fig. 2C and D), new bone trabeculae were observed fulfilling all the portions, from the base to the apex, in conjunction with a large amount of haematopoietic tissue. One specimen of the fresh-frozen group and one of the DFDBA group showed foreign-body reaction.

Intergroup comparisons for newly formed bone and remaining material in the apical and base portions are showed in Figure 3. In the apical portion, the fresh-frozen and DFDBA groups exhibited areas of new bone formation that were larger than that of the autogenous group ($p<0.05$). There was no significant difference in the comparison between allograft groups. The blood clot group did not exhibit new bone formation in this portion. In the basal portion, all the grafts showed areas of new bone formation greater than that observed for the blood clot group ($p<0.05$); however, the allograft group maintained the highest values compared with the autogenous group ($p<0.05$). Regarding remaining material, the fresh-frozen group showed area percentage greater than that observed for the DFDBA group, both in the apical ($p<0.001$) and base portions ($p=0.002$).

Fluorescence Microscopy

To investigate the mineral apposition in relation to the time (in weeks), we analysed microscopically the incorporation of three fluorochromes into the bone matrix. Figure 4 shows the percentage of fluorescence area in the basal portion of the specimens presented in each group individually. In all the groups, the area of alizarin-derived fluorescence (administered after 6 weeks) is significantly lower than that of calcein-blue derived fluorescence (administered after 9 weeks) ($p<0.05$). All the groups showed high percentage of fluorescence derived from calcein blue, which was administered after 9 weeks'

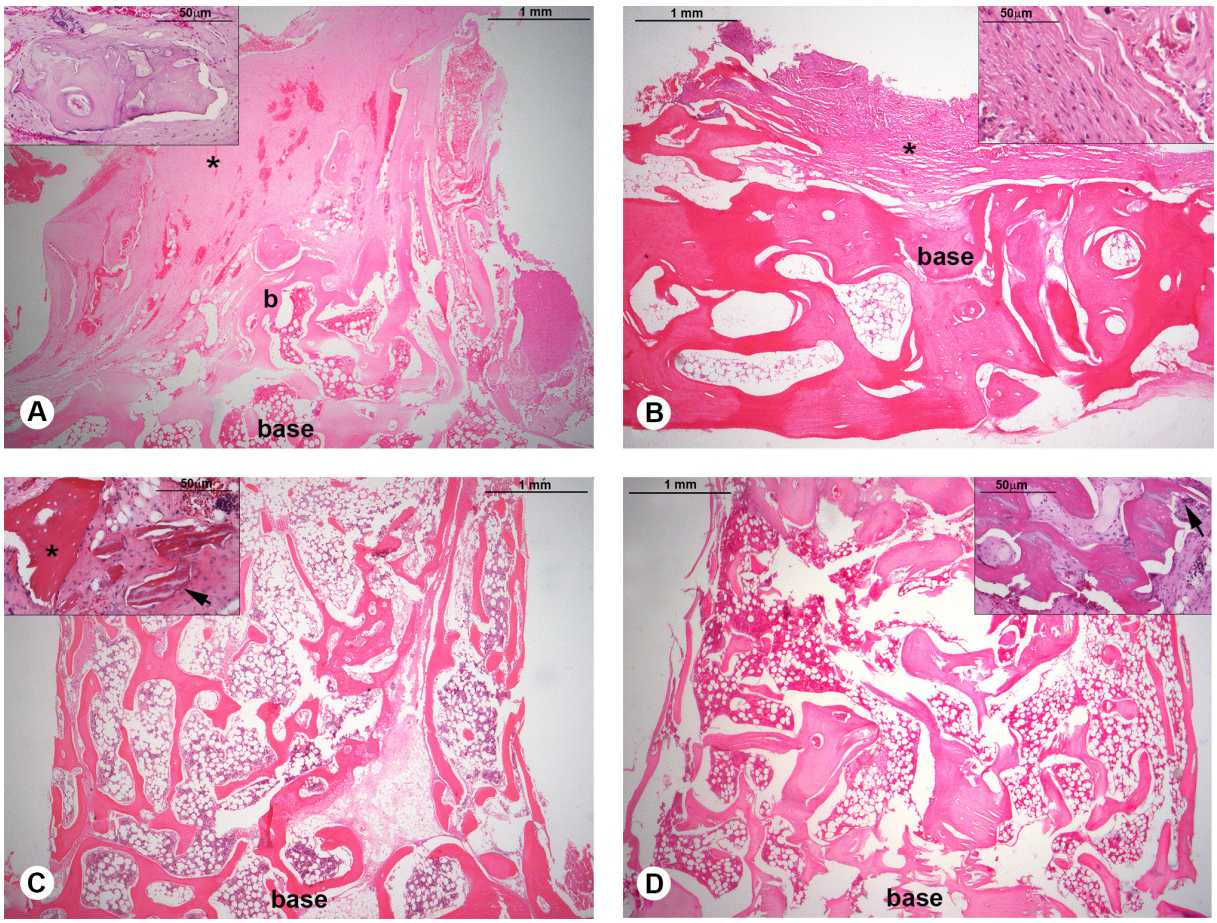

Figure 2. Representative histological sections for autogenous (A), blood clot (B), fresh-frozen (C), and DFDBA (D) groups (haematoxylin-eosin, 25X and 400X original magnification, scale bar = 1 mm and 50µm, respectively). A: Dense connective tissue in the apical portion (*) and new bone formation in the basal portion (b). In detail, isolated trabeculae surrounded by a dense connective tissue, showing immature bone. B: Connective tissue near from the base (*), and absence of new bone formation. In detail, connective tissue exhibiting high cellularity and dense collagenization. C: New bone trabeculae with hematopoietic tissue occupying the entire area of the cylinder. In detail, a fragment of fresh-frozen bone (*) at side of new trabeculae showing immature bone (arrow). D: Similar pattern to those observed in C, in which new bone formation is evident. In detail, new trabeculae showing high connectivity and immature bone, surrounded by osteoclast (arrow).

post-surgery, indicating a high mineral apposition at this experimental period. The autogenous group showed significant differences between alizarin-derived (6 weeks) and green calcein-derived fluorescence area (12 weeks) ($p<0.05$). Therefore, in general, the allografts did not alter the chronology of mineral deposition in the basal portion of the specimens in comparison with the autogenous and blood clot groups. In the apical portion (Fig. 5), the fresh-frozen and DFDBA groups showed more intense mineral deposition at 9 weeks compared with at 6 weeks ($p<0.05$); probably the allografts have intensified significantly the mineral deposition at 9 weeks. In the blood clot and autogenous groups, there was no significant difference between the periods in this portion of the specimens, probably due to the high variance of the data. Fluorescent probes are illustrated in Figure 6.

Discussion

Vertical augmentation in alveolar bone is difficult to achieve due to high rates of resorption in autograft bone. However, bone allografts have been demonstrated to be more effective as biomaterials at maintaining the bone volume for vertical tissue enhancement (1,7).

Although other studies assessed the differences in volume enhancement to correct bone vertical defects (15,16,20) the amount and dynamics of bone neoformation between allografts were not found in the literature comparing demineralised freeze-dried bone, mineralized fresh-frozen bone, allografts employing autogenous bone grafts.

We showed that DFDBA and FF exhibited larger averages in macroscopic and microscopic volumes of neoformed tissues compared with the autogenous bone. These results might be partially explained by the high rates of resorption exhibited by the autogenous bone when it was obtained using bone collectors, like others had previously shown (6). The negative result in control group (BC) indicates the osteoconductive characteristics of DFDBA, FF, or autogenous and their potential for tissue augmentation and maintenance comparatively achieved by onlay grafting techniques.

The vertical formed tissue appeared macroscopically stable from the base to the top of the cylinders, where they were adhered to the recipient bed, and exhibited blood vessels on their lateral walls. Although the necessity of making perforations in the cortex of the recipient bed

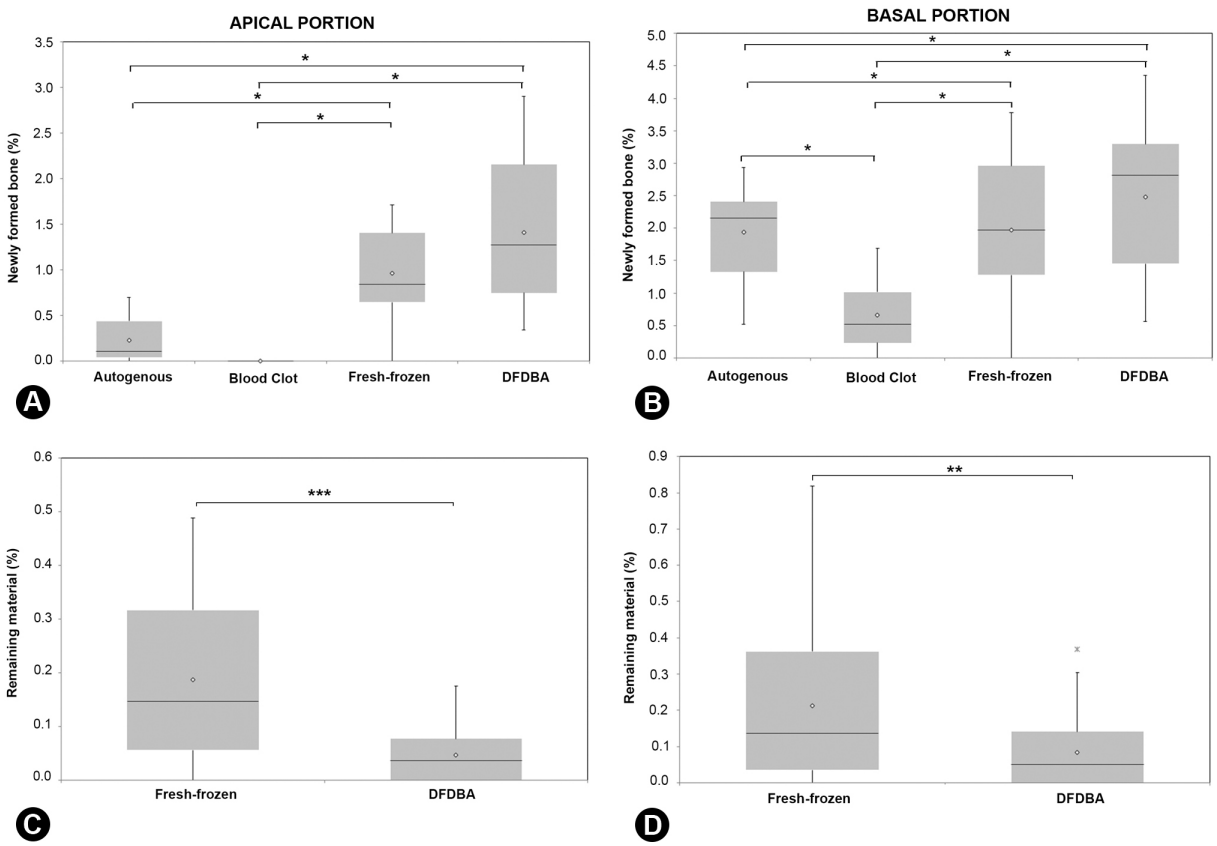

Figure 3. Box-plot for percentages of new bone formation (A and B) and remaining material (C and D) detected by histomorphometry in the apical and base portions of the defect. Line: median; ◊: mean; box limits: lower and upper quartiles; whiskers: minimum and maximum values; ○: outliers; *: $p<0.05$; **: $p<0.01$; *** $p<0.001$ (for Kruskal–Wallis' test followed by Dunn's test, and for Mann–Whitney test).

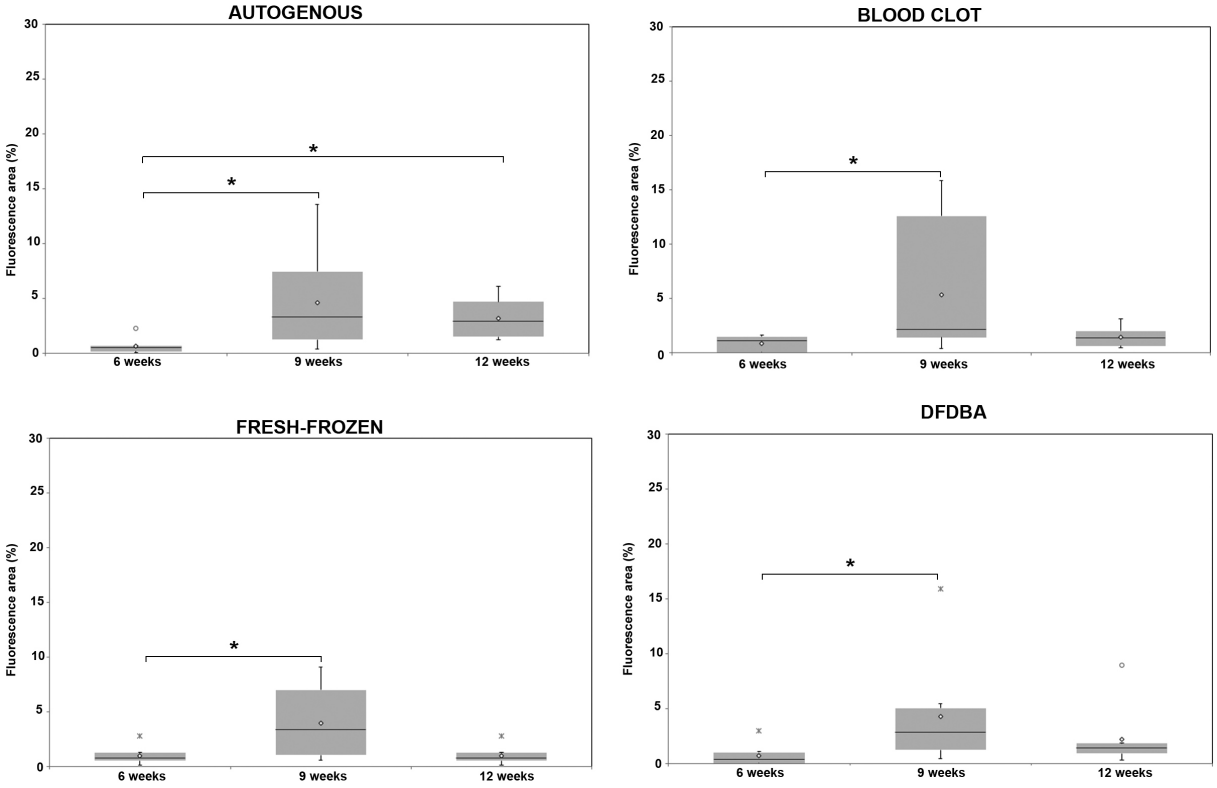

Figure 4. Box-plot for percentages of fluorescence area in the basal portion of the onlay newly formed tissue. Line: median; ◊: mean; box limits: lower and upper quartiles; whiskers: minimum and maximum values; ○: outliers; *: p<0.05 (for Kruskal–Wallis' test followed by Dunn's test).

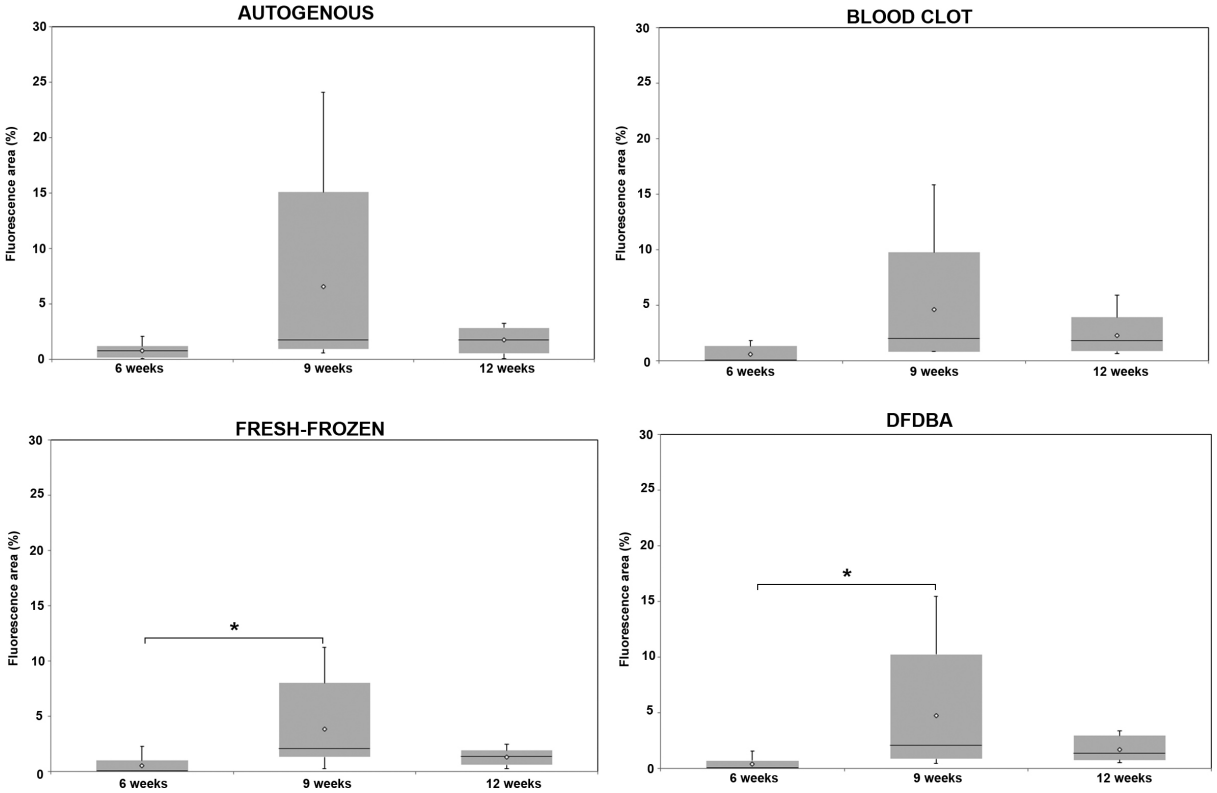

Figure 5. Box-plot for percentages of fluorescence area in the apical portion of onlay newly formed tissue. Line: median; ◊: mean; box limits: lower and upper quartiles; whiskers: minimum and maximum values; ○: outliers; *: p<0.05 (for Kruskal–Wallis' test followed by Dunn's test).

is controversial (19,20) we could observe, macroscopically, that all the samples showed visible blood vessels on lateral walls over onlay-formed tissue from all four interventions. Several authors have suggested that these perforations might allow for the entrance of osteogenic cells derived from the bone marrow and endosteum. That could promote blood clot formation and favour angiogenesis at the graft site, which are important features for GBR success (19,21).

Moreover literature shows some debates about ideal particle size of bone substitutes (22,23) certain authors (24) have demonstrated the importance of standardising the size of biomaterials particles. The present study rather wanted to simulate what would happen in routine clinical conditions of a surgical reconstruction, that is, using 500-µm granules for the DFDBA, reducing the FF using a bone grinder, and granulating the autogenous using bone collectors. Despite that fact, the size of the particles of FF and autogenous was similar.

The use of caps to close the titanium cylinders and the absence of a periosteum on top of the biomaterials might generate some debate because the periosteum is considered to play an indispensable role as a supplier of osteogenic cells and as blood nutrition of grafts (16,19,21). In addition, covering the biomaterials with a periosteum had proved difficult to achieve due to the cylinder dimensions and the lack of elasticity of periosteum tissue. Nevertheless, the findings of the present study corroborate those of other studies (15,16,21) that used the same animal model and that showed that bone formation did not depend exclusively on the presence of a periosteum.

A histological analysis from DFDBA and FF grafts samples comparatively presented more bone area formation than the other two interventions. Especially DFDBA showed the greatest area of vertical bone formation. The upper (apical) portion of the cylinders compared to the base portion showed minor new bone formation in all interventions; nevertheless, the DFDBA and FF graft interventions presented the major mean areas of bone formation in this portion, suggesting the enhanced osteoconductive potential of these grafts. This fact can be explained also when we consider that in a vertical model like this, with a titanium cylinder guiding bone vertical grown, stimulus for bone grown came rather from the base of the fixed cylinders, from the perforated cortical of the calvarias. When we ponder about bone grafting to fill bone cavities or to gain volume in sinus lifting new bone vascularization and regeneration will come from all borders and defect walls.

Samples of DFDBA and FF grafts presented remnants

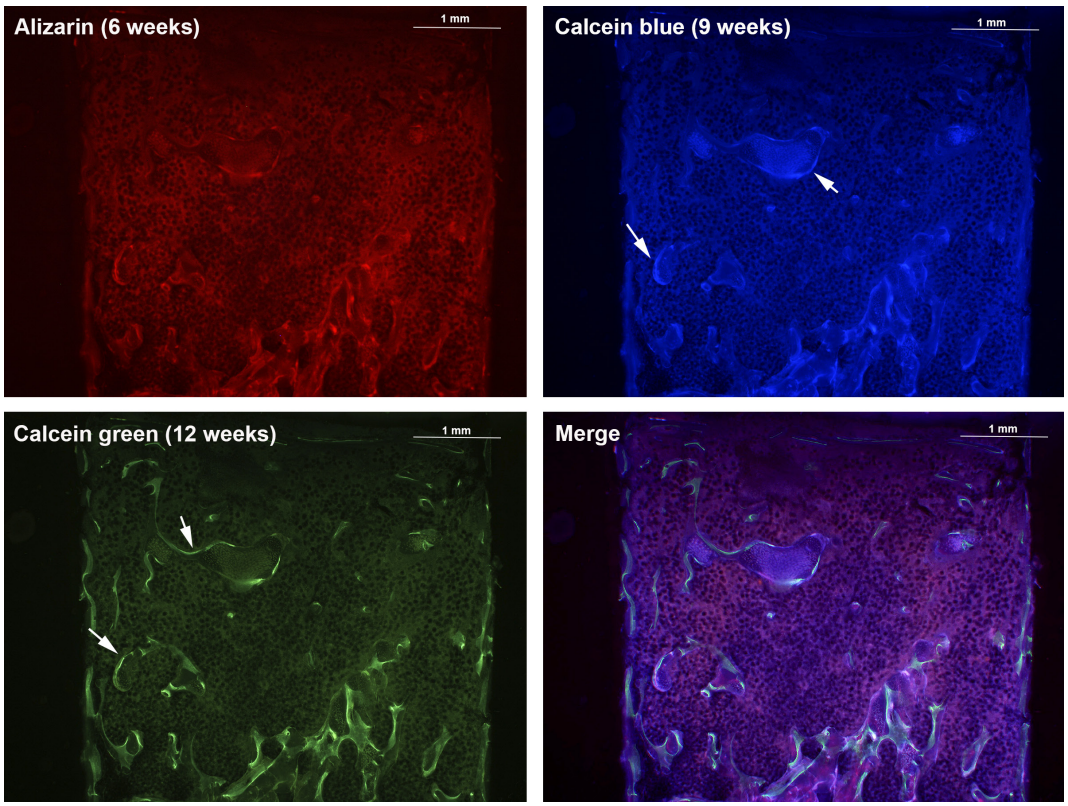

Figure 6. Representative histological sections showing the fluorescence pattern observed for each fluorochrome, injected at 6, 9, and 12 weeks. High area of fluorescence at 6 weeks is observed in the apical portion of the cylinder (arrows). At 12 weeks, high area of bone is noted, but the area of fluorescence is lower than that observed at 6 weeks (arrow heads).

within the onlay-formed tissue compared to the other two interventions. Although we detected remnants of allograft material in the histological analysis, the frequency of foreign body reaction was very low, and the interference on the haematopoietic tissue seemed to be minimal, suggesting a good biocompatibility of the materials.

Allograft bone basically acts as a support for new bone deposition. The replacement process in which the grafted bone endures progressive resorption is slow (23). Structural characteristics such as dimension of graft particles or chips are known to influence the amount of resorption and speed of new bone formation.

Due to this slow particle resorption, some authors emphasise the relevance of processing in reducing immunogenic reaction that could be provided by allografts (24,25). Although in the past some cross-infection has been reported from transplantation of allografts, new high standard protocols of screening tests for donors, irradiation, and antimicrobial procedures expressively diminish antigenicity (26).

Fluorescent bone markers allowed the quantification of bone mineralisation in different moments of new bone formation (18). All the groups seemed to form new bone at the same speed considering the basal portion of the specimen, with a peak of mineral apposition at 9 weeks after the surgery. However, in the apical portion, only allografts DFDBA and FF exhibited more mineral apposition at 9 weeks compared to 6 weeks, which suggests an improvement in bone deposition and remodelling by the allografts. Despite this augmentation on mineral apposition, demineralised and frozen bone allografts generally are associated with a poor mechanical resistance (25). Conversely to orthopaedic surgery, in the great majority of oral reconstructive surgery, there is no need for high biomechanical resistance at the early stage of bone repair. Therefore, the use of DFDBA and fresh-frozen bone could be indicated for fulfilment of bone defects in the oral cavity or in sinus lifting augmentation procedures, since they improved the velocity and the quantity of bone deposition in distant portions of the basal plane of the bone.

In continental countries where great climate discrepancies are expected, the transport of ultra-frozen bone tissue from collection and processing sites can compromise the success level of grafting procedures in distant locations. Accordingly, a great clinical relevance of lyophilised grafts is their availability, because they can be transported in ambient temperature without losing their biological properties. This situation may make DFDBA more attractive to use than fresh-frozen bone allograft because DFDBA is more easily stored, offering lower risk of contamination during transport (26).

In conclusion, the use of bone allografts, particularly DFDBA represents a reliable substitute for autogenous bone in the correction of volume bone defects because it showed a higher amount of new bone formation. In addition, it is safer easy to store, it is widely available at musculoskeletal tissue banks, it reduces surgical morbidity by eliminating the need for donor sites and shortens surgery times, thus favouring postoperative comfort.

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Resumo

Várias técnicas foram propostas para regeneração óssea vertical, e muitas delas usam enxertos ósseos e alogênicos ósseos. O objetivo deste estudo foi comparar os aloenxertos ósseos congelados desmineralizados (DFDBA), os aloenxertos congelados frescos (FF) com os enxertos ósseos autógenos para encontrar diferenças entre o volume, a histologia da formação óssea e a dinâmica do crescimento ósseo vertical. Um modelo ósseo de regeneração tecidual vertical foi realizado em calvarias de coelho sob anestesia geral. Quatro cilindros ocos de titânio puro foram parafusados nas calvarias de osso cortical externo em oito coelhos. Cada um dos cilindros foi preenchido aleatoriamente com uma intervenção: DFDBA, FF, osso autógeno ou com coágulo sanguíneo (BC) como controle. Os enxertos alogênicos foram obtidos a partir de um nono animal seguindo protocolos internacionais padronizados para a coleta, processamento e criopreservação de aloenxertos. O enxerto autógeno foi obtido da raspagem do fêmur do hospedeiro antes de adaptar os cilindros ocos. Os animais foram eutanasiados após 13 semanas. O volume vertical foi calculado após a medição, por meio de sonda milimetrada, do novo tecido formado dentro dos cilindros e após a remoção dos cilindros de titânio. Histomorfometria e coloração com fluorocromios foram utilizados para analisar a quantidade e a dinâmica da formação óssea. Os resultados mostraram que DFDBA e osso fresco congelado melhoraram a velocidade e a quantidade de deposição óssea em porções distantes do plano basal de enxerto. O material remanescente nos grupos de aloenxerto foi mais intenso do que em grupo autógeno. Ambos os aloenxertos podem ser indicados como alternativas confiáveis para ganho de volume e aumento ósseo vertical.

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