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Remodeling of cortical and corticocancellous fresh-frozen allogeneic block bone grafts – a radiographic and histomorphometric comparison to autologous bone grafts

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Abstract

Objectives: To compare cortical (AL-C) and corticocancellous (AL-CC) fresh-frozen block bone allografts to cortical block bone autografts (AT) used for lateral ridge augmentation in terms of radiographic dimensional maintenance and histomorphometrical graft remodeling.

Materials and methods: Twenty-four patients, requiring ridge augmentation in the anterior maxilla prior to implant placement, were treated with AT, AL-C or AL-CC bone blocks (eight patients per graft type). Patients were examined with CBCT prior to, 14 days, and 6–8 months after grafting. Amount of augmentation and dimensional block graft maintenance over time was evaluated by comparing planimetric measurements of the alveolar ridge made on CBCT sections of the augmentation area. During implant installation surgery, 6–8 months after grafting, cylindrical biopsies were harvested perpendicularly to the lateral aspect of the augmented alveolar ridge. The relative volumes of vital and necrotic bone and soft tissues were histomorphometrically estimated. Comparisons among groups and observation times were performed using Friedman test followed by Dunn's post-hoc test.

Results: Radiographic evaluation showed that the three types of grafts resulted in a significant increase in alveolar ridge width, with no significant differences among the groups in terms of ridge dimensions at the various observation times. However, significant graft resorption ($P = 0.03$) was observed in the AL-CC group over time ($-8.3 \pm 7.1\%$) compared with the AT and AL-C groups, where a slight increase was observed, on average ($1.5 \pm 20.6\%$ and $1.3 \pm 14.9\%$, respectively). Histomorphometrical analysis showed that larger amounts of vital bone were found in the biopsies from the AT augmented sites ($25.1 \pm 11.2\%$) compared with AL-CC and AL-C augmented sites ($9.3 \pm 3.8\%$ and $3.9 \pm 4.6\%$, respectively; $P \leq 0.01$). AL-CC and AT biopsies had the smallest amount of necrotic bone ($38.2 \pm 12.1\%$ and 56.7 ± 26.0 , respectively) compared with AL-C ($83.7 \pm 10.8\%$, $P < 0.01$) biopsies. AL-CC biopsies showed the largest amount of soft tissues ($52.5 \pm 11.7\%$) compared with those from AT ($18.1 \pm 17.1\%$, $P = 0.03$) and AL-C ($12.3 \pm 8.5\%$, $P < 0.01$) sites.

Conclusions: AL block bone graft architecture influences significantly its dimensional incorporation and remodeling. Compared with AT bone graft, a small portion of the AL block consists of vital bone 6–8 months after grafting. Cortical AL blocks seem to show the least amounts of vital bone, while corticocancellous AL blocks seem to undergo more resorption over time.

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Among the important factors connected to high success rates achieved in dental implant treatment is the presence of an adequate local bone volume (Stanford 2002). Nevertheless, the clinical reality is that, in many cases, the amount of available bone is reduced in patients who seek implant treatment. In those cases, a bone graft may be a

predictable way to restore bone volume and enable oral rehabilitation using dental implants (Chiapasco et al. 2006).

Autologous bone (AT) is still considered the gold standard graft material for bone augmentation procedures in the maxillo-facial region (Misch & Misch 1995; Nowzari & Aalam 2007). However, due to absence of

adequate volume of intraoral donor bone in cases of severe jaw resorption and with the purpose to avoid extra bone harvesting and the associated drawbacks (donor site morbidity and extra surgical time), other bone materials are frequently used (Goldberg & Stevenson 1994). Fresh-frozen allogeneic bone grafts (AL) have lately received renewed attention (Carinci et al. 2010; Hou et al. 2005; Lee et al. 2010; Spin-Neto et al. 2013b) due to the fact that recent strict processing guidelines have increased the safety of the material, practically eliminating the risk of patient cross-contamination (e.g., with hepatitis or HIV) (Waasdorp & Reynolds 2010), in contrast to practices of the past. Cases of infection, including hepatitis and HIV, due to implantation of contaminated allograft bone have indeed been previously observed (Salvucci 2011). So far, no major systemic complications have been reported after the use of AL grafts processed according to the new guidelines (Hinsenkamp et al. 2012), and recent studies have shown unremarkable immune reactions connected to AL-grafting of a magnitude relevant for alveolar ridge augmentation in humans (Spin-Neto et al. 2012, 2013a).

AL grafts exert primarily an osteoconductive property (Mizutani et al. 1990), acting as a scaffold into which host cells migrate, proliferate, differentiate, and produce new bone. It has been observed that AL exhibit slower incorporation and remodeling than AT grafts (Spin-Neto et al. 2013b; Spin-Neto et al. 2014; Waasdorp & Reynolds 2010). Previous preclinical *in vivo* studies have demonstrated that remodeling of an AT block bone graft depends also on variations in graft architecture, specifically the cortical-to-cancellous bone ratio (Ozaki & Buchman 1998). It is currently unknown whether the same is valid for AL grafts.

Therefore, the aim of this study was to compare cortical (AL-C) and corticocancellous (AL-CC) fresh-frozen block bone allografts to cortical block bone autografts (AT) used for lateral ridge augmentation in terms of radiographic dimensional maintenance and histomorphometrical graft remodeling, 6–8 months after grafting for anterior maxillary ridge augmentation.

Materials and methods

The protocol for this controlled case series was approved by the Araraquara School of Dentistry Ethics Committee (CEP-FO/Car) and by the National Research Ethics Com-

mittee (CONEP-MS) under the protocol number 36/08, and it is in accordance to the World Medical Association Declaration of Helsinki (2002). All treatments were performed in the Department of Diagnosis and Surgery, Section of Periodontology, Araraquara Dental School (UNESP – Univ. Estadual Paulista), Araraquara, São Paulo, Brazil.

Patient selection

A total of 40 partially or totally edentulous patients (23 female/17 male, age average: 45 years; range 18–69 years) who desired oral rehabilitation with dental implants and had at least one site with severe bone deficiency (i.e. <4 mm alveolar ridge width) impeding the placement of a regular size implant, were treated from May 2009 to May 2012. Alveolar ridge width was determined in cross-sectional image sections of Cone Beam Computed Tomography (CBCT, i-CAT Classic, Imaging Sciences International, Hatfield, PA, USA) generated images (DICOM-based data sets) with a resolution of 96 dpi, 14-bits gray scale and 0.25 mm voxel size. The CBCT unit was set at 120 kVp, 5 mA, with a 20 s. exposure time. None of the patients presented with systemic diseases affecting bone turnover, or were pregnant or lactating, or had habits that could interfere with treatment (for example: smoking, alcoholism and drug use).

In this case series, treatment group allocation (AL or AT) was not randomly assigned and was based on the following hierarchical criteria: (i) subjective estimation of augmentation needed, based on the clinical and radiographic (CBCT) screening examination and the amounts of donor bone available; patients with inadequate amounts of donor bone were allocated to AL, (ii) patients with adequate amounts of donor bone, but refusing a second surgical area for AT block graft harvesting, were thereafter allocated to AL; (iii) patients allocated to AL were then treated with AL-CC or AL-C block bone graft (only one type of graft in each patient irrespective of the number of blocks), depending on availability of the grafting material in the specific bone-bank (UniOss, Marília, Brazil). Accordingly, thirteen patients were treated with autologous cortical bone grafts (20 bone blocks; AT group), 19 patients were treated with fresh-frozen allogeneic corticocancellous bone grafts (52 bone blocks; AL-CC group), and eight patients were treated with fresh-frozen allogeneic cortical bone grafts (20 bone blocks; AL-C group).

To fulfill Brazilian regulations, documents regarding the allogeneic biomaterial request

were filled-out and sent to the registered bone bank that supplied the allografts (UniOss). The fresh-frozen allogeneic bone was collected and processed according to the American Association of Tissue Banking guidelines (Tomford 2000) and delivered at the University Clinic on the day before surgery. The AL-CC material was retrieved from the femoral head and/or patella, while the AL-C material was retrieved from the tibia.

In the present analysis, a matched-group design at the block level was used, as follows: the block from which the trephine biopsy was harvested in one randomly (by means of a lottery draw) chosen patient of the AL-C group was matched with the corresponding biopsied block placed in the same region (anterior maxilla) in one randomly chosen patient (with the same or a larger number of blocks) from each of the AT and AL-CC group. Thus, at the end, the analysis included eight patients/blocks per treatment group. Table 1 shows sample characteristics.

Surgical procedures

Immediately prior to the augmentation surgery, a mouth rinse was performed with 10–15 ml 0.12% Chlorhexidine digluconate for 1 min, and Povidine-iodine 10% solution was applied to the peri-oral skin. Then, under local anesthesia (Mepiadre 2%, DFL, São Paulo, Brazil), a full thickness flap was raised to provide full visualization of the resorbed alveolar ridge. All remaining soft tissues were removed from the bone surface and small-size round burs (ZB-70 Carbide bur; MicroAire, Chicago, IL, USA) were used under intense saline solution irrigation to penetrate the host cortical bone for enhancing vascularization toward the base of the grafts. In the AT group, cortical grafts of adequate size according to defect dimensions were retrieved from the mandibular ramus. In the AL-CC and AL-C groups, standard size bone blocks (15 × 10 × 6 mm – AL-CC; 15 × 10 × 4 mm – AL-C) were removed from the freezer and put into sterile saline solution for at least 10 min, allowing them to hydrate and obtain room temperature gradually. The blocks were trimmed and compressed using burs, under abundant sterile saline solution irrigation, to match the defects. The trimming and compression procedures deliberately reduced the amount of soft tissue (medulla) in the AL-CC blocks, but always largely preserving their corticocancellous architecture. After compression, AL-CC blocks showed a thickness comparable to AT and AL-C blocks, ranging from 3.5 to 4.5 mm, as measured with a caliper. AL-CC

Table 1. Characteristics of the matched study sample

Triplet patient group	Region	Group AT			Group AL-CC			Group AL-C		
		Age (years)	Gender (F/M)	CBCT Sections	Age (years)	Gender (F/M)	CBCT sections	Age (years)	Gender (F/M)	CBCT sections
1	Anterior maxilla	46	F	10	39	F	10	46	M	8
2	Anterior maxilla	21	M	6	44	F	6	50	F	13
3	Anterior maxilla	37	M	7	42	M	9	61	F	11
4	Anterior maxilla	33	F	9	57	F	12	55	F	7
5	Anterior maxilla	57	F	9	50	F	13	38	F	10
6	Anterior maxilla	43	F	8	32	F	10	56	F	8
7	Anterior maxilla	47	M	9	31	M	8	39	M	10
8	Anterior maxilla	54	F	7	55	F	10	56	F	8
Averages/Totals		42	5/3	8.1	44	6/2	9.7	50	6/2	9.4

blocks were fixed with the cancellous bone side facing the host bone. All bone grafts were fixated using 1.5 mm × 10 or 12 mm (Ø × length) titanium screws (Neodent, Curitiba, Brazil) and were covered with a collagen membrane (Genius Baumer, São Paulo, Brazil). Then, the flaps were repositioned to cover the grafts completely and sutured with interrupted nylon 4-0 single sutures.

All patients received antibiotics (Amoxicillin 500 mg) three times daily for 7 days, non-steroidal anti-inflammatory treatment (Nimesulide 100 mg) two times daily for 5 days, and analgesics (Acetaminophen 750 mg) according to individual needs. Patients were advised to rinse with Chlorhexidine digluconate for the following 7 days. Sutures were removed 14 days after surgery.

CBCT image section assessment

In addition to the pre-treatment screening scanning, all patients were scanned again 14 days- (14 d) and 6 months (6 m – AT and AL-CC groups) or 8 months (8 m – AL-C group) after grafting (prior to implant installation) using the same standardized exposure parameters. The DICOM data sets were saved on a hard disk and reconstructed using specific software (OnDemand 3D 1.0.7.0295, Cybermed, Seoul, South Korea) to reconstruct the image sections and export the 2-dimensional image sets for the quantitative evaluation of bone dimensions.

Only the data sets obtained at 14 d and 6 or 8 m were used for analysis, as image section generation from pre-surgical CBCT scans could not be performed in a standardized manner and with the same spatial orientation due to absence of a fixed reference (i.e. the fixation screw). In the post-surgical scans, the graft fixation screw was used as a guide to re-orientate the data sets from the two time-points, so that sections with the same spatial orientation could be generated for both time-points (Spin-Neto et al. 2013c). Thus, 0.25 mm thick cross-sections (distance

between sections = 1 mm) through the bone block and the residual ridge and entire jawbone were generated parallel to the long axis of the screw and perpendicular to the occlusal plane, and extended throughout the entire bone block width. Consequently, the number of sections varied among blocks depending on individual width; the same number of sections was generated and evaluated for each particular block for both observation periods. The contrast of the images was adjusted, and the center level (L) and band-width (W) were set according to the suggestions in the software (W = 3086 and L = 667).

For every CBCT section, one TIF (tagged image file) image was generated. The images had a resolution of 96 dpi matching the scanner resolution and therefore avoiding any distortion. They contained a ruler, which allowed the setting of the linear scale of each image. All generated images were anonymized and randomized, to mask the evaluator with respect to treatment group and observation period.

The assessment of the TIF images was performed using dedicated software (ImageJ, NIH, Bethesda, MD, USA) by manually tracing the structures under study with the computer mouse. To attain a measuring standard in the images, the total maxillary bone area (TBA_{Final}, in mm²) visible in each image section was included and traced, as presented elsewhere (Spin-Neto et al. 2013c). In the 14 d images, TBA (TBA_{14d}) was estimated by

adding the traced areas (Fig. 1) of the grafted bone block and the host bone. The changes in bone area (CBA) over time (from 14 d to 6 or 8 m) were calculated and expressed as a percentage of bone area at 14 d.

Bone biopsies: clinical procedures and evaluation

Implant installation surgery was performed six (AT and AL-CC) or eight (AL-C) months after the grafting procedure. During this session, one cylindrical biopsy, including the graft and part of resident bone, was retrieved perpendicularly to the lateral aspect of the augmented ridge by means of a trephine bur (2 mm internal Ø) from each patient.

The biopsies were routinely fixed, decalcified, dehydrated, embedded in paraffin, and sectioned. Three 6 µm thick hematoxylin-eosin stained sections, representing central aspects of the cylindrical biopsy, were used for histological and histomorphometrical analysis using a DIASTAR light microscope (Leica Microsystems, Wetzlar, Germany) connected to a Leica Microsystems DFC-300-FX digital camera (Leica Microsystems). A standardized (6 × 2 mm, length × width) area of interest (AOI), was digitally over-projected on the most lateral (buccal) part of the biopsy; thus the AOI represented 55–75% of grafted bone and 25–45% of resident bone for the various groups. The relative amount (%) of vital bone (VB), necrotic bone (NcB), and soft tissues (ST) within grafted and resident bone

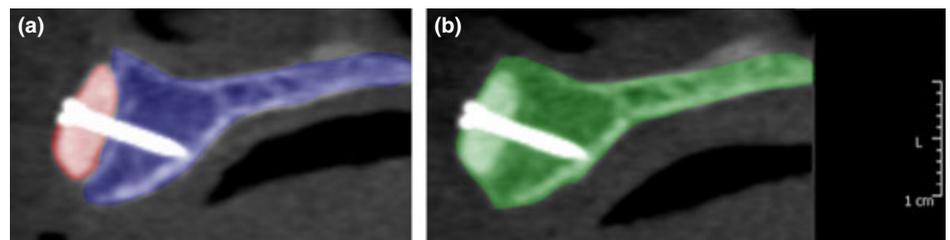


Fig. 1. Example of the measured areas in each image. At 14 d (a), the host bone (in blue) and the grafted bone block (in red) areas were separately measured. At 6 m (b), the total bone area (“TBA_{Final}”, in green) was measured.

in the AOI was planimetrically measured using the ImageJ software (NIH).

Data analysis

Commercially available software (GraphPad Prism 6.0 for Windows, GraphPad Software Inc., La Jolla, CA, USA) was utilized to compare all assessed parameters and draw graphics. Due to the limited sample size and to avoid random statistical differences, data was treated as not normally distributed. For the radiographic evaluation, comparisons among groups regarded the mean total bone area assessed on all sections of each block (TBA_{14d} and TBA_{Final}), and the % changes in bone area (CBA) between the two observation periods. For the histomorphometric evaluation, comparisons regarded the relative amount (%) of vital bone (VB), necrotic bone (NcB), and soft tissues (ST) within AOI. Friedman test (due to the repeated measures, with the block graft as the repeated variable) followed by Dunn's post-hoc test for multiple comparisons. The level of significance was set at $P < 0.05$.

Results

Clinical observations

No complications were observed throughout the study and all block bone grafts showed radiographic and clinical signs of incorporation to receptor bed at 6 or 8 m post-operatively. All planned implants could subsequently be inserted.

CBCT evaluation

The results of the CBCT evaluation are presented in Table 2. Host bone was statistically similar for all groups. A significant increase in TBA was observed from baseline ("Host Bone Area") to 14 d and 6 m and 8 m ($P \leq 0.05$) in all groups, but there were no statistically significant differences among AT, AL-C, and AL-CC groups regarding TBA at both observation times. The grafted bone block area observed in the 14 d images was larger in the AL-CC group compared to the two other groups

($P = 0.04$ and 0.02 , for AL-C and AT, respectively). The TBA_{Final} was not different from TBA_{14d} in all groups. Regarding % CBA, AL-CC showed statistically significant larger bone area loss when compared to both AT ($P = 0.03$) and AL-C ($P = 0.03$) groups.

Histological and histomorphometric evaluation

Areas of necrotic bone of varying size with empty osteocyte lacunae and absence of capillaries (in the Haversian canals) were consistently observed in all biopsies. The interface between resident bone and the bone block graft was not generally distinct for either the AT or AL-CC groups, while it was easier to identify in the AL-C group. The newly formed bone was in direct contact with – or occasionally completely engulfed within necrotic bone and only a few osteoclasts were seen in the sections. The marrow spaces in the AL-CC blocks were primarily filled with dense fibrous connective tissue and bone marrow was only occasionally observed, irrespective of the group. There were no signs of inflammatory process in any of the groups. Figure 2 shows a representative histological section of each group.

The results of the histomorphometric evaluation are presented in Table 2 and Fig. 3. The AL-CC ($P = 0.003$) and AT ($P = 0.01$) groups showed the smallest amount of necrotic bone, compared with the AL-C groups. AL-CC also showed significantly larger amounts of soft tissues than AT ($P = 0.03$) and AL-C groups ($P = 0.002$). In contrast, statistically significant larger amounts of vital bone were found in the AT group, compared with both AL-CC ($P = 0.006$) and AL-C groups ($P = 0.003$). AL-CC and AL-C groups were statistically equal regarding amounts of vital bone.

Discussion

Various aspects of the treatment outcome, including the radiographic maintenance and histological remodeling of the block grafts, of

the AT and AL-CC patients included herein, have been reported in previous papers (Spin-Neto et al. 2013b,c; Spin-Neto et al. 2014). Inclusion of data from those patients aimed to allow comparisons with the AL-C treated patients, which are presented for the first time in this study. The analysis included eight patients/blocks per treatment group, allowing the matching of both radiographic and histological assessments. The use of fresh-frozen allogeneic bone (AL) block grafts has increased in the last decades, primarily because the implementation of strict guidelines for donor selection and material processing, which has practically eliminated the risk for disease transmission (Waasdrop & Reynolds 2010). Thus, AL has recently also gained attention for intraoral indications, in cases where large volumes of augmentation are needed and there is limited availability of intraoral AT (Spin-Neto et al. 2013b).

The results of the radiographic assessment showed that all types of grafts resulted in a significant increase in alveolar ridge dimensions, but also showed that corticocancellous AL block bone grafts (AL-CC) presented significantly larger dimensional reduction (ca. -8.5%) than cortical AL (AL-C) or autogenous (AT) block bone grafts, which showed a slight increase (ca. $+1.5\%$), 6–8 months post-surgery. The observed reduction was most likely primarily due to volumetric changes in the AL-CC grafted block itself and not due to vast changes of the alveolar process. The large dimensional reduction associated with AL-CC grafts observed herein has been already discussed in a previous report on all patients included in the AL-CC and AT groups of the present patient material (Spin-Neto et al. 2014). At that point, it was suggested that the reduction of AL-CC in comparison to the AT blocks (herein, being mostly cortical) could partly be explained by differences in graft architecture, in terms of cortical-to-cancellous bone ratio. The present results, in which similarly low amounts of dimensional reduction were observed in the

Table 2. Average \pm standard deviation (range) for radiographic host bone area, graft block bone area, total bone area (TBA) at the various observation periods, and % change in bone area (CBA), and for histologic relative volumes of vital bone, soft tissues, and necrotic bone in the AT, AL-CC, and AL-C groups

Parameter	AT	AL-CC	AL-C
Host bone area (mm ²)	182.9 \pm 62.2 (123.2–301.4) ^a	136.4 \pm 51.3 (75.3–215.0) ^a	178.7 \pm 35.4 (134.6–239.2) ^a
Grafted bone block area (mm ²)	27.5 \pm 7.4 (16.3–37.0) ^a	56.8 \pm 17.0 (24.4–75.5) ^b	29.4 \pm 10.4 (12.8–44.3) ^b
TBA _{14d} (mm ²)	203.3 \pm 63.3 (136.0–308.6) ^a	193.5 \pm 63.9 (105.5–271.8) ^a	204.6 \pm 29.6 (172.8–264.8) ^a
TBA _{Final} (mm ²)	215.5 \pm 76.9 (115.47–322.72) ^a	181.2 \pm 55.8 (91.0–244.6) ^a	211.0 \pm 37.8 (160.8–262.8) ^a
CBA% ("Average")	1.5 \pm 20.6 (–32.7–4.0) ^a	–8.3 \pm 7.1 (–15.4–4.0) ^b	1.3 \pm 14.9 (–23.8–22.2) ^a
Vital bone	25.1 \pm 11.2 (11.8–40.9) ^a	9.3 \pm 3.8 (2.8–13.4) ^b	3.9 \pm 4.6 (0.2–13.3) ^b
Soft tissues	18.1 \pm 17.1 (1.8–52.8) ^a	52.5 \pm 11.7 (30.3–67.5) ^b	12.3 \pm 8.5 (1.3–24.6) ^a
Necrotic bone	56.7 \pm 26.0 (17.4–84.0) ^{ab}	38.2 \pm 12.1 (26.3–59.6) ^a	83.7 \pm 10.8 (67.1–96.6) ^b

Same letters indicate non-significant differences between the groups, for that specific parameter determined factor.

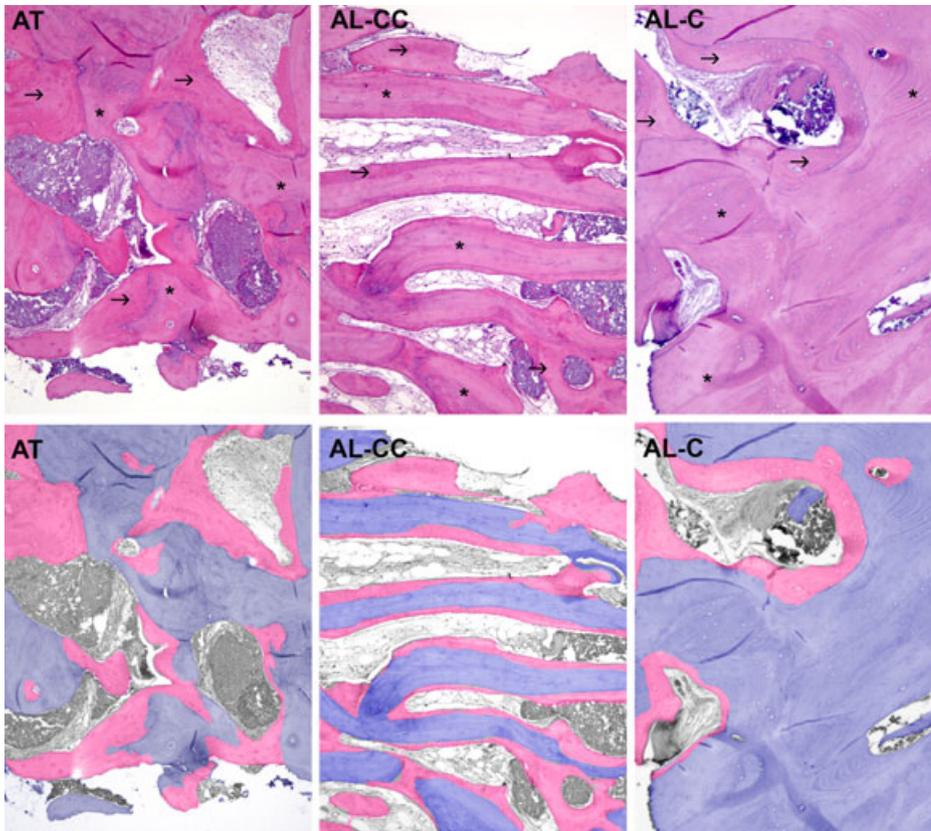


Fig. 2. In the first row, photomicrographs of characteristic biopsies retrieved from AT, AL-CC, and AL-C augmented ridges. New bone (→) in direct contact with necrotic bone (*) was observed in all three types of grafts. No signs of inflammatory reaction or excessive osteoclastic graft resorption could be observed, irrespective of graft type. In the second row, the same images are shown with masked colors, representing vital bone (red), necrotic bone (blue) and soft tissues (gray).

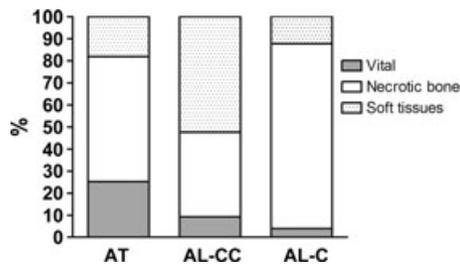


Fig. 3. Stacked percentages of vital bone, necrotic bone, and soft tissues percentage in the biopsies (mean%), for all groups.

AL-C and AT blocks compared with the AL-CC blocks (which also showed the highest amount of soft tissues with the grafted area), corroborate the above assumption and indicate that variations in bone block graft architecture in terms of cortical-to-cancellous bone ratio may be more important than the origin (AL or AT) of the graft itself, with regard to the dimensional maintenance of the block. The assumption that bone block architecture is important for the dimensional maintenance of an AL block bone graft is in accordance with current understanding of AT bone block graft remodeling and incorpora-

tion. Reports from preclinical *in vivo* studies evaluating the influence of AT bone block architecture on block graft maintenance, remodeling and incorporation have indeed shown that cancellous and corticocancellous AT block bone grafts present significantly larger resorption compared to cortical AT bone blocks (Goldstein et al. 1995; Ozaki & Buchman 1998; Ozaki et al. 1999).

On the other hand, although the AL-C blocks presented volumetric maintenance similar to that observed for the AT cortical blocks, the histologic analysis in the present study showed significant differences in terms of remodeling of the two types of bone block. The AL-C bone blocks remained almost entirely necrotic after 8 months of healing and showed minimal amounts (4%) of vital bone only in the proximity of the resident alveolar ridge. In contrast, 25% of a biopsy from an AT cortical block consisted of vital bone. Thus, despite the fact that implants were successfully placed in the planned position and subsequently loaded in all patients included in the present study, no assumptions on the long-term prognosis of the treatment can be made. Some recent short-term

studies have suggested that AL may show results similar to those achieved with AT grafts, regarding the possibility of implant installation in the augmented sites (Carinci et al. 2010, 2009a,b; Waasdorp & Reynolds 2010). However, it is important to interpret those results carefully, as implant success rates of only 40% after 4 years were reported, due to the increased rate of peri-implant marginal bone loss at implants inserted in AL augmented sites (Carinci et al. 2010, 2009b). In perspective, the longitudinal success rates for implants placed in AT augmented ridges are in general >90% (Chiapasco et al. 2009). The finding that all implants placed in AL augmented sites in the present group of patients did osseointegrate and could be loaded may be explained by the fact that the implants were inserted primarily within resident bone and within the narrow inner aspect of the block graft containing a large proportion of vital bone. Nevertheless, it must be kept in mind, that the biological and biomechanical challenges, on the long-term, of a loaded largely non-vital block graft remain unknown. In addition, the results from a recent preclinical *in vivo* study suggest that peri-implantitis may progress faster when the peri-implant tissues contain non-vital bone biomaterials compared with cases where implants are installed in pristine sites (Stavropoulos et al. 2012). The histological observations in the present analysis, showing basically non-vital bone toward the lateral (buccal) aspects of the AL blocks, may explain the above-mentioned increased peri-implant marginal bone resorption reported in the literature (Carinci et al. 2010, 2009b).

The histomorphometric analysis in the present study showed that biopsies from the AL-CC blocks presented smaller amounts of necrotic bone compared to those from the AL-C and AT blocks (37%, 84%, and 58%, respectively), although this difference is significant only for the AL-C group. No differences in terms of vital bone were observed between the two types of AL blocks, and the smaller amount of necrotic bone in the AL-CC originated in the fact that the mineralized bone fraction in this type of blocks was initially lower than in the AL-C blocks, due to its trabecular structure. Earlier studies have attributed the reduced incorporation/remodeling of AL (when compared to AT) to the possible presence of antigenicity in AL (Kirkeby et al. 1992; Stevenson et al. 1991). However, AL grafting in the present group of patients did not result in IL-10, IL-1b, IFN-c, and TNF-a serum values beyond the

physiological levels (Spin-Neto et al. 2012), indicating that AL grafting does not seem to challenge significantly serum cytokines. Thus, the reduced remodeling/incorporation of AL grafts must be attributed to some other factors than antigenicity, currently not well understood. Nevertheless, the reduced remodeling/incorporation of AL block grafts may be simply due to absence (largely) of bone cells surviving the processing to initiate bone apposition/resorption (creeping substitution), as is the case with AT grafts (Roberts & Rosenbaum 2012).

In conclusion, AL block bone graft architecture influences significantly its dimensional

remodeling and incorporation. Compared to AT bone grafts, only a small portion of the AL block consists of vital bone 6–8 months after grafting. Cortical AL blocks seem to show the least amounts of vital bone, while corticocancellous AL blocks show the highest amount of soft tissues and seem to undergo more resorption over time. It seems thus reasonable to suggest that AL-C and AL-CC bone block grafts may be an option only in cases where a relatively limited amount of augmentation is needed and where the future implant can be predictably positioned primarily within resident bone and the inner aspects of the block showing

large amounts of vital bone. However, the possibility of peri-implantitis, peri-implant bone loss, and implant failure in the long-term could be a concern more relevant for AL than AT grafts, due to the small quantities of viable bone found on the augmented site.

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