Pattern of osteogenesis during onlay bone graft healing

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Objective. The aim of this paper was to analyze how healing occurs between onlay bone graft and the mandible cortex.
Study design. Autologous and allogeneic corticocancellous bones, harvested from the ilium wing, were grafted at each mandible side of 40 rabbits. One side received platelet-rich plasma (PRP). Killings occurred at 3, 7, 14, 28, and 56 days. Tissues were stained by hematoxylin-eosin and toluidine blue. New bone area was measured at different regions of sections stained with toluidine blue. Wilcoxon test was used to analyze differences among regions and Bonferroni test toanalyze the influence of PRP, graft nature, and days.
Results. Osteogenesis was higher at the lateral region ($P < .05$). PRP tended to improve bone neoformation, which was higher at the allogeneic graft. Statistical significance among the different categories of variables—grafts, use of PRP, and days of observation—did not have a linear behavior. A linear behavior of statistical tests was not detected. Bone new formation increased until the 14th day ($P < .05$).
Conclusions. Onlay grafts heal due to osteogenesis which occurs at the lateral region and between the cortex and host mandible. Allogeneic grafts and PRP tend to improve bone formation. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;110:713-719)**

The correct placement of osteointegrated implants plans that recipient bone presents sufficient bone height and thickness.[1] Onlay bone grafts were introduced to achieve the proper thickness of the edentulous alveolar ridge. These grafts need to induce osteoconduction to guarantee its healing in the recipient bed.[2-6] The final result of these reconstructions is often satisfactory. However, onlay grafts on the jaws are invariably subject to some degree of resorption.[7,8] To ensure these grafts' healing and to minimize their resorption, they have been associated with platelet-rich plasma (PRP).[9,10]

Despite the large use of this graft technique, works that investigate the healing biology of the grafts and its incorporation to the jaws are rare.[11-15] Therefore, it becomes relevant to investigate how healing of onlay bones grafted to the mandible occurs. Bone secretion at the interfaces between the graft and the recipient bed was also considered.

MATERIALS AND METHODS

Surgical procedure

The protocol of this work is in accordance with the Ethics Committee for Use of Animals of the Bahia Foundation for Science Development in Salvador, Bahia, Brazil. Forty nonisogenic albino male New Zealand rabbits, weighting 2.5-3 kg were used. Two groups of 20 animals were constituted. In one group, rabbits received fresh autologous grafts, and in the other, animals were grafted with allogeneic bone previously frozen at −70°C for 120 days.

For surgery, atropine (2 mg/kg) was administered. The anesthesia was conducted with acepromazine (1 mg/kg) and ketamine (10 mg/kg). Enrofloxacin (10 mg/kg) was used for antibiotic prophylaxis. After trichotomy and asepsis of all surgical regions, 2 mL bupivacaine (0.5%) with adrenaline (1:200.000) were infiltrated into the iliac region and 1.5 mL into the mandible. From the wing of the right ilium, a bicortical

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circular block was harvested with a trephine bur of 1 cm external diameter.

Bones harvested were sheared into 2 corticocancellous fragments through the cancellous space. Each corticocancellous fragment was measured and positioned in one side of the mandible. Bones were grafted in such way that the cancellous portion was placed toward the recipient mandible. They were fixed to the mandible with a titanium screw through the lag screw technique.

On the left side, no adjuvant was added between the graft and the soft tissue, whereas on the right side, 500 μL PRP was placed between the graft and its overlying soft tissue. In each group, 5 subgroups of 4 animals each were constituted. These subgroups were differentiated by the time until their killing, which occurred after 3, 7, 14, 28, and 56 days.

PRP preparation

Nine milliliters of total blood was harvested through cardiocenthesis using a needle containing 1.5 mL ACD-a as anticoagulant (JP Farmacêutica, São Paulo, Brazil). Whole blood was centrifuged (Biofuge Stratos; Haereus Inst., Osterode, Germany) in a relative centrifuge acceleration (RCA) of 300*g* for 10 minutes at a constant temperature of 22°C. The plasma and 1 mm of the red cells interface were harvested and recentrifuged at an RCA of 5,000*g* for 5 minutes at a temperature of 22°C. The coagulation of the PRP was achieved with an addition of 15 μL 10% calcium chloride per 500 μL of PRP. PRP platelet count was measured with an automated hematology analyzer.

Analysis of the neoformed bone area

After killing, a mandible fragment containing the recipient bed, the graft, and the soft tissue over them was removed. Tissues were fixed in 10% buffered formalin for 72 hours and decalcified in 10% tetrahydrated EDTA, 1 mol/L, pH 7.2, for 90 days. Tissues were subjected to standard histotechnical processing and were embedded in paraffin so that, at the sections, the recipient bed, the graft, the soft tissue, and its interfaces could be visualized. Slides, stained with hematoxylin and eosin, were used to study bone graft general behavior. The bone neoformation was analyzed through toluidine blue stain under histomorphometric measurement of the areas most strongly impregnated by the dye. The neoformed matrix area was measured on the lateral parts of the graft, on its interface with the recipient bed and with the soft tissue, and at the turnover of the bone matrix of the trabeculate and the graft cortex.

Statistical analyses

Mean ± SD of PRP platelet count were calculated. Student *t* test analyzed the difference on the amount of platelets that autologous and allogeneic grafts received. One-way analysis of variance (ANOVA) was the test used to evaluate the differences in graft volume among groups. The difference in area of neoformed bone in each region was analyzed by the Wilcoxon test. An analysis on each day of observation was performed. At this analysis, the behavior of each type of graft, autologous or allogeneic, was evaluated at each side of the mandible with or without PRP. Also, the contribution of PRP was investigated considering each type of graft. The influence of time in relation to the osteogenic potential was also studied. The statistical difference between the categories of the variables, including the effect of time, was evaluated by the Bonferroni test. All significance levels considered were 5%.

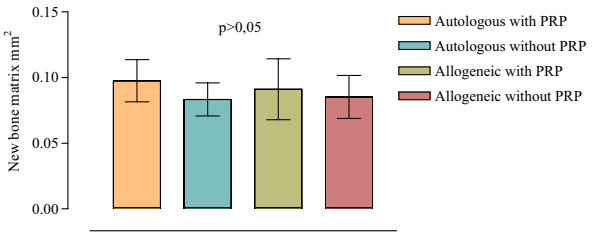

Fig. 1. Volume of corticocancellous bone grafted for each group studied.

RESULTS

Platelet count mean ± SD in the PRP was 2,345,930 ± 921,610/μL (autologous: 52.75 ± 4.84; allogeneic: 46.05 ± 6.40; $P = .183$). Platelet concentration in the PRP was 6.6 ± 2.3–fold that of whole blood. All groups received a similar volume of corticocancellous bone (autologous with PRP: 0.097 ± 0.016 mm^3; autologous without PRP: 0.083 ± 0.012 mm^3; allogeneic with PRP: 0.091 ± 0.023 mm^3; allogeneic without PRP: 0.085 ± 0.016 mm^3; $P > .05$; Fig. 1).

Onlay graft consolidation at the host mandible occurs due to bone osteoconduction below the graft cortex. Bone deposition at graft extremities also assures graft integration to the mandible (Fig. 2). Very little neoformation was achieved between the cortex graft and the soft tissue above it (Fig. 3). The graft matrix was gradually absorbed and a new matrix secreted, characterizing graft turnover. This pattern of bone secretion was observed in every rabbit regardless of whether they received autologous or allogeneic bone or PRP. Inflammatory cells were more intense at the 3rd day; they were a result of the surgical procedures. There was no cell infiltration in any rabbit that resembled a reaction of the host to the graft (Fig. 4).

New bone formation was higher at the lateral region of the onlay graft, followed by the neoformation be-

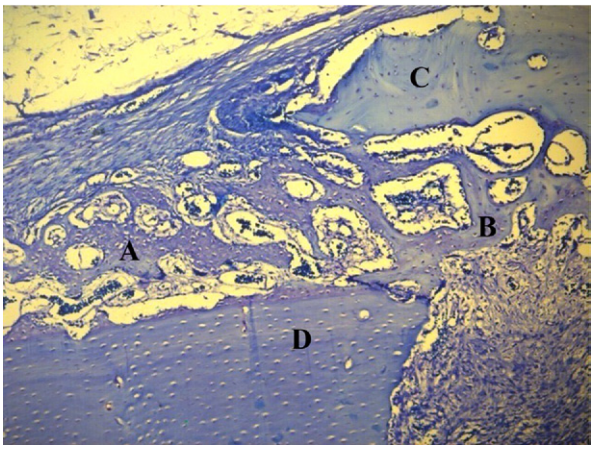

Fig. 2. Osteogenesis at the lateral region (A) and between (B) the cortex of the onlay graft (C) and the host mandible (D). Allogeneic graft with platelet-rich plasma, 28th day. Toluidine blue stain, ×400.

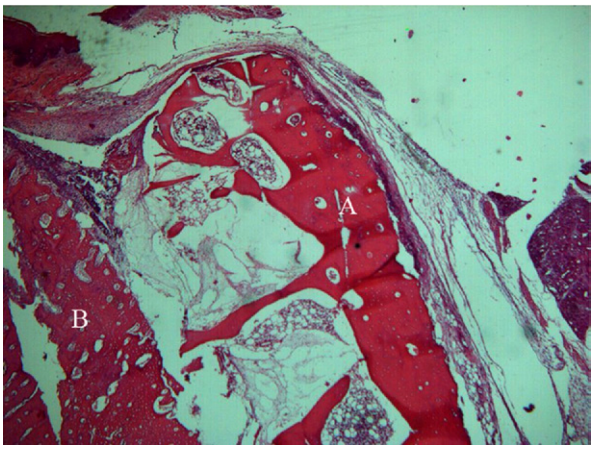

Fig. 4. Region between the graft (A) and the recipient bed (B). Allogeneic graft with platelet-rich plasma, 3rd day. Hematoxylin-eosin stain, ×400.

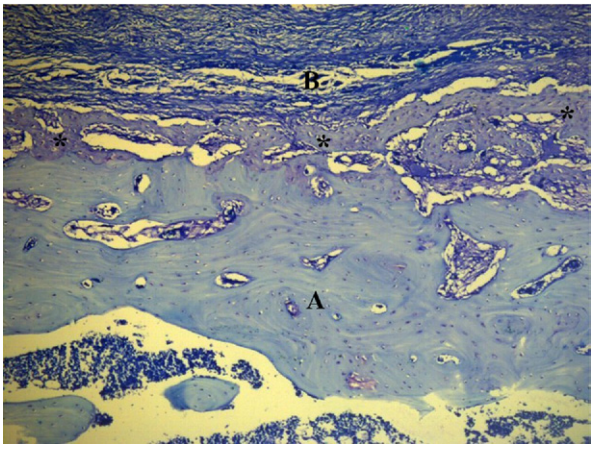

Fig. 3. Osteogenesis (*) between the superior region of the onlay graft (A) and the soft tissue (B). Autologous graft without platelet-rich plasma, 14th day. Toluidine blue stain, ×400.

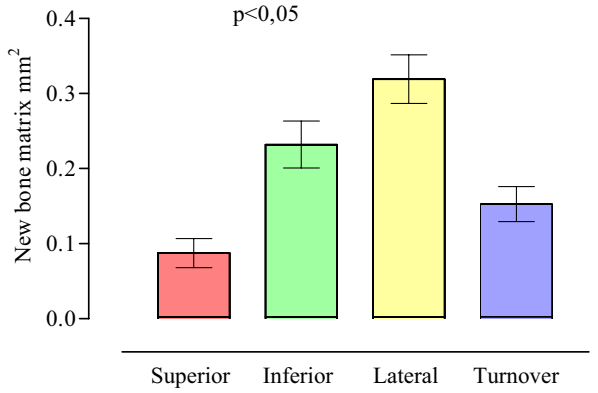

Fig. 5. Osteogenesis at the different areas of the onlay grafts.

tween graft cortex and mandible cortex. The comparison between the 4 areas showed that the difference among them was statistically significant ($P < .05$; Fig. 5).

Difference between the studied groups at each of the observation days

3rd day. Autologous grafts, compared with allogeneic ones, in each side of the mandible, tended to induce a higher area of osteogenesis at superior (Table I) and lateral (Table II) regions, on the side where PRP was added. However, at the inferior region (Table III), allogeneic bone was better on the side without PRP. Turnover was minimal at this moment (Table IV). If PRP effect was analyzed according to each graft type, this concentrate of growth factor had a tendency to increment osteogenesis, independently of the nature of the bone grafted. Nevertheless, at this day, no difference had statistical significance ($P > .05$).

7th day. Autologous graft seemed to induce more bone matrix secretion in the lateral and inferior regions ($P > .05$; Tables II and III). In the superior region, the higher osteogenesis was attributed to allogeneic grafts at the side without PRP ($P < .05$; Table I). Minimal modifications at the original graft matrix took place at this day (Table IV). Osteogenesis attributed to PRP was obvious at the superior region (Table I) around allogeneic grafts ($P < .05$; Table I).

14th day. In the inferior region (Table III), autologous grafts exhibited more matrix than allogeneic grafts. In the superior and lateral regions (Tables I and

Table I. Area of bone neoformation at the superior portion of the onlay graft according bone nature and platelet-rich plasma (PRP) use, along the different days of experiment

	Neoformed bone area (mm^2)—superior region			
	Autologous graft		*Allogeneic graft*	
Period	*PRP*	*No PRP*	*PRP*	*No PRP*
03 days	0.075 ± 0.12	0.044 ± 0.10	0.061 ± 0.05	0.058 ± 0.08
07 days	0.031 ± 0.08	0.086 ± 0.16	0.069 ± 0.08[a]	0.199 ± 0.54[a]
14 days	0.241 ± 0.21[a,c,d]	0.004 ± 0.01[a,b]	0.213 ± 0.49[a,c,d]	0.012 ± 0.03[a,b,c,d]
28 days	0.101 ± 0.13[e]	0.004 ± 0.02[e]	0.131 ± 0.33[e]	0.002 ± 0.01
56 days	0.077 ± 0.21[e]	0.028 ± 0.08[e]	0.062 ± 0.17[e]	0.091 ± 0.14[e]

Values are presented as mean ± SD. Significance is determined by Bonferroni test.
[a]$P < .05$ for the differences between PRP use or not for each graft.
[b]$P < .05$ for the differences between graft natures at each side.
[c]$P < .05$ for the difference between the 3rd day and the others at each side of mandible and each graft.
[d]$P < .05$ for the difference between the 7th day and the others at each side of mandible and each graft.
[e]$P < .05$ for the difference between the 14th day and the others at each side of mandible and each graft.

Table II. Area of bone neoformation at the lateral portion of the onlay graft according bone nature and platelet-rich plasma (PRP) use, along the different days of experiment

	Neoformed bone area (mm^2)—lateral region			
	Autologous graft		*Allogeneic graft*	
Period	*PRP*	*No PRP*	*PRP*	*No PRP*
03 days	0.192 ± 0.26	0.030 ± 0.35	0.057 ± 0.05	0.028 ± 0.03
07 days	0.146 ± 0.78	0.116 ± 0.24	0.167 ± 0.19	0.103 ± 0.09
14 days	0.749 ± 0.63[a,c,d]	0.288 ± 0.21	0.574 ± 0.44[c]	0.514 ± 0.21[c]
28 days	0.317 ± 0.27[e]	0.027 ± 0.03[b]	0.685 ± 0.73[c,d]	0.581 ± 0.32[b,c,d]
56 days	0.230 ± 0.25[c,d]	0.257 ± 0.22[c,d,e,f]	0.376 ± 0.29	0.335 ± 0.22

Values are presented as mean ± SD. Significance is determined by Bonferroni test.
[a]$P < .05$ for the differences between PRP use or not for each graft.
[b]$P < .05$ for the differences between graft natures at each side.
[c]$P < .05$ for the difference between the 3rd day and the others at each side of mandible and each graft.
[d]$P < .05$ for the difference between the 7th day and the others at each side of mandible and each graft.
[e]$P < .05$ for the difference between the 14th day and the others at each side of mandible and each graft.
[f]$P < .05$ for the difference between the 28th day and the others at each side of mandible and each graft.

II), this phenomenon only happened in the presence of PRP. Turnover (Table IV) tended to be more intense around autologous grafts if PRP was not present ($P > .05$). In general, bone matrix secretion was higher in the presence of PRP, especially in the lateral region (Table II) around autologous graft and in the superior region around both grafts ($P < .05$).

28th day. Allogeneic grafts were more osteogenic than autologous ones at the lateral and inferior regions of bone (Tables II and III; $P < .05$). Matrix turnover (Table IV) and matrix deposition in the superior region (Table I) on the PRP side had a tendency to be higher around autologous bone ($P > .05$). Around the inferior region (Table III) of allogeneic grafts, PRP significantly increased bone secretion ($P < .05$). Around all other regions, it also tended to occur if PRP was inserted at surgical bed ($P > .05$).

56th day. Although differences were not significant ($P > .05$), allogeneic grafts induced more matrix deposition in the lateral and inferior regions (Tables II and III) and in the superior region (Table I), if PRP was not present. Turnover (Table IV) was higher with autologous bone. Similarly, PRP contributed to a higher turnover ($P > .05$; Table IV) and more bone matrix synthesis in the inferior region (Table III; $P > .05$). In the superior region (Table I), such input occurred around autologous grafts and at the lateral region (Table II) around allogeneic grafts.

Effect of time on the onlay graft

In the superior area, time exerted the same influence on autologous and allogeneic grafts if they were associated with PRP. For both grafts, a significant growth in matrix area was observed between the 3rd and 14th days ($P < .05$) and between the 7th and 14th days ($P < .05$). The 28th and 56th days presented lower bone secretion than the 14th day ($P < .05$). On the side without PRP, osteogenesis was present at the 3rd, 7th,

Table III. Area of bone neoformation at the inferior portion of the onlay graft according bone nature and platelet-rich plasma (PRP) use, along the different days of experiment

	Neoformed bone area (mm^2)—inferior region			
	Autologous graft		*Allogeneic graft*	
Period	*PRP*	*No PRP*	*PRP*	*No PRP*
03 days	0.030 ± 0.04	0.067 ± 0.13	0.096 ± 0.03	0.053 ± 0.07
07 days	0.136 ± 0.20	0.111 ± 0.11	0.110 ± 0.08	0.085 ± 0.10
14 days	0.621 ± 0.41[c,d]	0.498 ± 0.33[c,d]	0.489 ± 0.26[c,d]	0.268 ± 0.28
28 days	0.115 ± 0.18[b,e]	0.023 ± 0.04[b,e]	1,046 ± 0.62[a,b,c,d,e]	0.387 ± 0.44[a,b]
56 days	0.119 ± 0.20[e]	0.052 ± 0.09[e]	0.137 ± 0.26[f]	0.089 ± 0.12

Values are presented as mean ± SD. Significance is determined by Bonferroni test.
[a]$P < .05$ for the differences between PRP use or not for each graft.
[b]$P < .05$ for the differences between graft natures at each side.
[c]$P < .05$ for the difference between the 3rd day and the others at each side of mandible and each graft.
[d]$P < .05$ for the difference between the 7th day and the others at each side of mandible and each graft.
[e]$P < .05$ for the difference between the 14th day and the others at each side of mandible and each graft.
[f]$P < .05$ for the difference between the 28th day and the others at each side of mandible and each graft.

Table IV. Turnover at the onlay graft according bone nature and platelet-rich plasma (PRP) use, along the different days of experiment

	Neoformed bone area (mm^2)—matrix turnover			
	Autologous graft		*Allogeneic graft*	
Period	*PRP*	*No PRP*	*PRP*	*No PRP*
03 days	0.006 ± 0.01	$1 \times 10^{-19} \pm 1 \times 10^{-21}$	$1 \times 10^{-19} \pm 1 \times 10^{-21}$	$2 \times 10^{-20} \pm 2 \times 10^{-21}$
07 days	0.004 ± 0.01	$6 \times 10^{-19} \pm 2 \times 10^{-21}$	$8 \times 10^{-20} \pm 2 \times 10^{-21}$	$8 \times 10^{-20} \pm 2 \times 10^{-21}$
14 days	0.105 ± 0.29	0.498 ± 0.20	0.119 ± 0.18	$8 \times 10^{-20} \pm 2 \times 10^{-21}$
28 days	0.351 ± 0.49[c,d]	0.369 ± 0.28	0.315 ± 0.39	0.161 ± 0.19
56 days	0.434 ± 0.17[c,d,e]	0.339 ± 0.26[c,d]	0.374 ± 0.41	0.171 ± 0.20[c,d,e,f]

Values are presented as mean ± SD. Significance is determined by Bonferroni test.
[c]$P < .05$ for the difference between the 3rd day and the others at each side of mandible and each graft.
[d]$P < .05$ for the difference between the 7th day and the others at each side of mandible and each graft.
[e]$P < .05$ for the difference between the 14th day and the others at each side of mandible and each graft.
[f]$P < .05$ for the difference between the 28th day and the others at each side of mandible and each graft.

and 56th days. Therefore, little new released bone matrix was present at the 14th and 28th days. The significance of these differences are presented in Table I.

At the lateral and inferior regions, osteogenesis increased until the 14th day around autologous grafts. Around allogeneic grafts, the area of new bone matrix increased until the 28th day. After these days, osteogenic potential gradually decreased. This behavior happened whether PRP was added or not. Tables II and III show, for these regions, the significance of the differences among the groups according to the days of observation.

Except for the autogenous graft and the side where PRP was added, all others groups' turnover (Table IV) progressively increased along the periods of observation. However, osteogenesis, in general, was more intense from the 14th day. Around autologous grafts, the 28th day was more osteogenic than the 3rd and 7th ($P < .05$), and the 56th day more than the 3rd, 7th, and 14th days ($P < .05$) on the PRP side. On the side without PRP, only the 56th day differed from others ($P < .05$). Around allogeneic bone, significance only appeared for the difference between the 56th day and others ($P < .05$) on the PRP side.

DISCUSSION

Onlay grafts represent an excellent alternative for jaw thickening.[7,16] Nevertheless, the healing biology of the bone grafted onto the alveolar ridge using this technique is rarely discussed in the literature. Works that report this technique are limited to case reports.[3,4,16] Therefore, the methodology proposed in the present research contributes in a relevant manner to a better knowledge about a common procedure on maxillary rehabilitation.

The results obtained allowed the conclusion that consolidation of onlay corticocancellous grafts occurs due to new bone formation on the periphery of the graft and inside the cancellous portion between the cortex and the recipient bed.

These data are consistent with the biologic principle that the cortex of the graft functions as an important biologic membrane for the matrix deposition below it.[17,18]

Despite the osteoconduction at graft edges, the occurrence of this phenomenon on the superior region of the graft was very low. Probably, the proliferation of the soft tissue in close contact with the onlay graft occurred in a more exuberant way than the differentiation of mesenchymal cells in osteoblasts and subsequent bone matrix secretion.[19,20] Regarding the turnover, it is also possible to state that, due to the neoformation that occurred in the interior of the cortex and trabecules, the biology of the onlay grafts predicts that most of the grafted bone is gradually replaced by new bone.[21]

The technique of onlay grafting is widely disseminated, with the innovations recently introduced of incorporating PRP in the protocols and the use of allogeneic bone. PRP, as an autologous source of growth factors,[22] works in a way that stimulates a higher proliferation of preosteoblasts,[23] which could result in an increment of osteogenesis associated with onlay grafts. The frozen allogeneic graft, besides providing the benefit of avoiding surgery at a second area, presents a higher resistance to absorption.[24-26] Some authors advocate that owing to the denaturizing of cholesterol in the cancellous portion during freezing, the osteoconduction potential of this type of bone graft would be even higher.[26,27]

The suppositions about PRP can be confirmed by the data obtained in the present research. For the most part in the experiment, the addition of PRP had a tendency to contribute with an increment in bone neoformation. Little statistical significant differences were noticed, though.

This performance is concordant with the inferences obtained by analysis of the literature regarding PRP.[19,28-30] This globular concentrate is an excellent mitogen and induces a higher secretion of cellular products. However, it is poor in inducing cell differentiation.[23,31-35] The production of the matrix would be increased by the osteoblasts already present on the tissue, whereas preosteoblasts would be stimulated to remain in mitosis. The finalization of the differentiation process that would be necessary to enhance bone amount, would only occur at a later moment.[36] Therefore, these arguments suggest that PRP could not be able to induce an exuberant bone synthesis.

Regarding the role of PRP in the present experiment, it is important to highlight the osteogenesis observed in the superior region on the 14th day around the autologous and allogeneic grafts. At that time, the little osteogenesis that occurred was only possible because of the presence of PRP. On the sides without this source of growth factors, neoformation was practically nil.

On the final days of the investigation, allogeneic bone still presented an osteogenic potential in almost all areas, slightly higher than autologous bone. This type of graft preserved for a longer time its capacity to induce osteoconduction, corroborating the findings of other experiments that suggested the superiority of this material.[37-39] Regarding the intrinsic osteogenic profile of the allogeneic graft, it is important to highlight that on the 56th day, the remaining osteogenesis was higher than the autologous bone in the superior, lateral, and inferior regions independently of PRP.

It is important to state that, regarding the progression of the days, the 14th day was the most osteogenic for the autologous graft at most regions studied. Allogeneic bone presented a similarity in the osteogenesis of the 14th and 28th days, concordant with what was observed regarding the property of this graft of keeping the osteoconduction active for a longer period of time.

Turnover of the cortex and cancellous portions of the bone occurred in a more relevant way after the 14th day of observation. This event began only after the peak of osteogenesis had occurred at the lateral and inferior regions. A minimum consolidation with the recipient bed was necessary so that the turnover could occur afterward.

CONCLUSION

It is possible to conclude that, independently of bone nature, this technique can thicken the alveolar ridge before implant placement. The graft consolidates in its recipient bed owing to the bone neoformation below its cortex and at its periphery. Despite the fact that PRP increases bone neoformation, the results do not allow us to infer linearity as to the benefit of its use.

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