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Osteointegration of autologous and allogeneic onlay grafts

Osteointegração de enxertos onlay autólogos e alogênicos

Osteointegración de injertos onlay autólogos y alogénicos

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Abstract
The aim this paper was investigate the biology of incorporation of autologous and allogeneic onlay grafts to mandible of rabbits in association or not with Platelet Rich Plama (PRP). Forty animals composed this experiment. Bones were harvested from the wing of ilium and onlay grafted bilaterally to mandible cortex. Half of the animals received fresh autologous bone and the others received allogeneic bone previously frozen under -70ºC for 120 days. On the right side of the mandible, PRP was added. Euthanasia of the animals took place after 3, 7, 14, 28 and 56 days. Histological sections were stained with hematoxilin and, eosin, toluidin blue, picro-sirius and TRAP. For immunohistochemical study, anti-CD79αcγ, anti-CD3 and anti-RAM11 antibodies were considered. At all variables studied, a superiority on the behavior of allogeneic grafts, especially in the latest periods of grafting was observed. The largest amount of macrophages was detected around allogeneic bones, despite it being the less absorbed. The presence of PRP did not modify the behavior of those variables in a statistically significant way. The frozen allogeneic bone represented a good material for the thickening of the jaw and did not induce immunological reaction. PRP was not effective in improving the healing of onlay bone grafts.
Keywords: Autografts; Transplantation, homologous; Bone regeneration; Bone transplantation; Cryopreservation; Platelet-rich plasma; Allergy and immunology.

Resumo
O objetivo deste trabalho foi investigar a biologia da incorporação de enxertos onlay autólogos e alogênicos em mandíbula de coelhos em associação ou não com Plama Rico em Plaquetas (PRP). Quarenta animais compuseram este experimento. Os ossos foram colhidos da asa do ílio e onlay enxertados bilateralmente no córtex da mandíbula.

Metade dos animais recebeu osso autólogo fresco e os demais receberam osso alogênico previamente congelado a -70ºC por 120 dias. No lado direito da mandíbula, foi adicionado PRP. A eutanásia dos animais ocorreu após 3, 7, 14, 28 e 56 dias. Os cortes histológicos foram corados com hematoxilina e, eosina, azul de toluidina, picro-sirius e TRAP. Para o estudo imuno-histoquímico, foram considerados os anticorpos anti-CD79αcγ, anti-CD3 e anti-RAM11. Em todas as variáveis estudadas, observou-se superioridade no comportamento dos enxertos alogênicos, principalmente nos períodos mais tardios de enxertia. A maior quantidade de macrófagos foi detectada ao redor dos ossos alogênicos, apesar de ser o menos absorvido. A presença do PRP não modificou o comportamento dessas variáveis de forma estatisticamente significativa. O osso alogênico congelado representou um bom material para o espessamento da mandíbula e não induziu reação imunológica. O PRP não foi eficaz em melhorar a cicatrização de enxertos ósseos onlay.
Palavras-chave: Autoenxertos; Transplante homólogo; Regeneração óssea; Transplante ósseo; Criopreservação; Plasma rico em plaquetas; Alergia e imunologia.

Resumen
El objetivo de este trabajo fue investigar la biología de la incorporación de injertos onlay autólogos y alogénicos a la mandíbula de conejos en asociación o no con Plaqueta Rica en Plaquetas (PRP). Cuarenta animales compusieron este experimento. Los huesos se extrajeron del ala del ilion y se injertaron de manera bilateral en la corteza mandibular. La mitad de los animales recibieron hueso autólogo fresco y el resto hueso alogénico previamente congelado a -70ºC durante 120 días. En el lado derecho de la mandíbula, se agregó PRP. La eutanasia de los animales tuvo lugar después de 3, 7, 14, 28 y 56 días. Las secciones histológicas se tiñeron con hematoxilina y eosina, azul de toluidina, picro-sirius y TRAP. Para el estudio inmunohistoquímico se consideraron anticuerpos anti-CD79αcγ, anti-CD3 y anti-RAM11. En todas las variables estudiadas se observó una superioridad sobre el comportamiento de los injertos alogénicos, especialmente en los últimos periodos de injerto. La mayor cantidad de macrófagos se detectó alrededor de los huesos alogénicos, a pesar de ser los menos absorbidos. La presencia de PRP no modificó el comportamiento de dichas variables de forma estadísticamente significativa. El hueso alogénico congelado representó un buen material para el engrosamiento de la mandíbula y no indujo reacción inmunológica. El PRP no fue eficaz para mejorar la cicatrización de los injertos óseos onlay.
Palabras clave: Autoinjertos; Trasplante homólogo; Regeneración ósea; Trasplante óseo; Crioconservación; Plasma rico en plaquetas; Alergia y inmunología.

1. Introduction

Despite the satisfactory results of the autologous and allogeneic bone grafting, prior to implant installation, the onlay bone is subjected to some degree of resorption.(Clayman, 2006) Aiming at assuring the regeneration of those grafts, minimizing its absorption and reducing the time from the grafting to the placement of the implants, several adjuvants have been proposed.(Thorwarth, et al. 2005) Among them, Platelet Rich Plasma (PRP)(Andrade, et al. 2008), an autologous platelet concentrate introduced in 1997, should be highlighted (Holly, & Mracna, 2009, Pessoa et al. 2009).

Although the wide use of onlay grafts and the great technical improvement observed in surgical protocols, papers that appreciate the biology and the chronology of the healing process of bone grafted by such technique and its incorporation to the jaws are scarce. Hence, it seems relevant to propose a discussion about the gaps that grew in parallel to the upgrading of the protocols for maxillaries grafting, as well as regarding the biological features of allogeneic grafts, and the possible contribution of PRP for graft incorporation.

2. Methodology

2.1 Surgical procedures

This research was performed in accordance to the criterion of the Ethics Committee for Animal Use and Care from according protocol number 02/2016. Forty non-isogenic, male, albine New Zealand rabbits weighing from 2.5 to 3.0 kg were used. Twenty animals received fresh autologous graft and the others were grafted with allogeneic bone previously frozen under -70o C for 120 days.

Before surgery, atropine (2mg/kg) was administered. Anesthesia was achieved with the use of acepromazine (1mg/kg) and ketamine (10mg/kg). Enrofloxacin (10mg/kg) was used for antibiotic prophylaxis. After shaving and asepsis, bupivacaíne 0.5% with adrenaline, 1:200,000 was infiltrated in all surgical beds. A bicortical circular block of bone was harvested from the

wing of the right ilium with a trephine burr of 1cm diameter. The block of bone was then divided in two cortico-cancellous fragments through the cancellous space. Height and weight of each cortico-cancellous fragment was measured.

Each cortico-cancellous fragment was onlay grafted on one side of the mandible with the cancellous portion placed toward the perforations. Before placing the graft, perforations were made at the cortex of the recipient bed. Grafts were fixed with titanium screw using the lag screw technique. On the right side, 500µl of PRP was inserted between the cortex surface of the recipient bed and the cancellous portion of the graft, and between the graft and the soft tissue. On the left side, no adjuvant was added. Two groups were considered, the autologous and the allogeneic bone, and each was subdivided in 5 subgroups, composed by 4 animals, considering the days elapsed from the surgery to the euthanasia, which occurred in the 3rd, 7th, 14th, 28th and 56th days.

2.2 Treatment of the grafts for placement in the recipient bed

Autologous bone was grafted at the recipient bed during the same surgical procedure performed for its harvesting. Bone fragments were immersed in 0.9% saline solution, during the time necessary to the divulsion of soft tissue around mandible. Allogeneic grafts were obtained from rabbits submitted to the autologous grafting, at the moment of euthanasia, soon after general anesthesia and before the excessive administration of the anesthetic drug. Such bone fragments were frozen under the temperature of -70º C for 120 days. Before placement in the recipient bed, they were washed with 100 ml of 0,9% saline solution and immersed in a 40% of gentamicin solution for 40 minutes.

2.3 Obtaining of PRP

Through cardiocenthesis, 9 ml of whole blood were harvested, in a syringe containing 1,5ml of ACD-A as anticoagulant (Pharmaceutical JP, São Paulo, Brazil). The centrifugations protocol used were proposed by Efeoglu, Akçay and Ertürk (18) and Andrade et al (10). Whole blood was centrifuged (Biofuge Stratos®, Haereus Inst, Osterode, Germany) under 300g for 10 minutes at 22ºC. Plasma was pipetted up to 1mm of the interface with the red cells and re-centrifuged under 5000g for 5 min at 22oC. PRP coagulation was obtained with the addition from 15µl of 10% chloride of calcium to 500µl of PRP.

2.4 Histological processing

After euthanasia, jaw fragments containing the graft and the soft tissue were obtained. The whole material was fixed in 10% buffered formalin during 48 hours. After this period, the soft tissue was carefully incised for screw removal. Decalcification was performed in 10%, 1M, EDTA pH 7.2. The material was processed and embeded in paraffin. The section was obtained so that the soft tissue, the graft and their interfaces with the recipient bed were visualized in the histological field. Sections of 5mµ were stained with hematoxilin and eosin (HE), toluidin blue, picro-sirius and the staining to evidence the tartrate resistant acid phosphatase (TRAP).

Immunohistochemical study was performed at 4mµ sections, assembled in slides treated with 6% organsilan. The primary antibody used for identification of B-lymphocytes was anti-CD79αcγ (clone HM59, Dako, 1:50, Hamburg, Germany). Anti-CD3 antibody was used to study T-lymphocytes, (policlonal, Dako, 1:100, Hamburg, Germany), anti-RAM11 (Dako, 1:100, Hamburg, Germany), for the investigation of macrophages. Antigenic retrieval was obtained in Tris-EDTA, pH 9.0, during 30 minutes, under humid heat. Endogenous peroxidase was blocked with two baths of hydrogen peroxide, and protein inhibitor (Dako, Hamburg, Germany) blocked unspecific reactions. Primary antibodies were incubated during 10 hours at 2°C and then tissues were incubated with secondary antibodies during 30 minutes at 2°C. The color of immunoreactions was achieved with diaminobenzidine (Dako, Hamburg, Germany). Hematoxilin was used for counter-staining. As positive control,

sections of rabbit spleen fixed at 10% buffered formalin were used. The negative control was the test tissue suppressed from the reaction with the primary antibody.

2.5 Hystological analysis

The histological evaluation was accomplished using Motic B5 Professional Series microscope. A descriptive analysis was performed according to the findings observed at the slides stained with HE.

Parameters of the digital images were quantified through Motic Image Advance 3.2. In the sections stained with HE, the thickness of the cortex graft and the total area of the bone matrix, regarding the graft and the neoformed bone, were quantified. In sections stained with the toluidin blue, the neoformed bone area was measured. With picro-sirius staining, the collagen matrix density at the soft tissue above the graft was quantified at areas measuring 0.25mm2. In the sections that evidenced the TRAP, osteoclasts behavior was analyzed.

2.6 Statistical analysis

The difference of surgery duration, height and thickness of graft cortex and PRP platelet count among groups were weighted by Student T-test. The statistical difference among the categories of the major variables was evaluated by Tukey's test. Considering that the distribution of the data differed from the Normal, Mann-Whitney's non-parametric test was used to determine the statistical difference of the graft type and the use of PRP on osteoclasts behavior. The non-parametric Kruskal-Wallis' test was used to analyze the effect of time on osteoclasts population. All significance levels considered were 5%.

3. Results

3.1 Surgical data

Animals grafted with autologous bone resembled to animals that received allogeneic grafts, according to the duration of the surgery (autologous, M±DP: 52.75±4.84; allogeneic, M±DP: 46.05±6,40; p=0.183), to the thickness (autologous, M±DP: 0.18±0.03; allogeneic, M±DP: 0.17±0,02; p=0.446) and to the width of the blocks grafted on the left side (autologous, M±DP: 0.90±0.01; allogeneic, M±DP: 0.90±0,02; p=0.548) as well as to the thickness (autologous, M±DP: 0.19±0.02; allogeneic, M±DP: 0.17±0,01; p=0.473) and to the width of the block of bone grafted on the right side (autologous, M±DP: 0.90±0.03; allogeneic, M±DP: 0.90±0.03; p=0.630). PRP platelet count indicated that both groups of grafts received similar amounts of platelets (autologous, M±DP: 2,171.95±973.79; allogeneic, M±DP: 2,519±855.25; p = 0.237).

3.2 Descriptive analysis

Healing of bone graft on the mandible cortex occurred throughout the days with the substitution of cancellous tissue for a mature trabeculate. There was no evidence of specific recognition of the graft by the immune system.

In the first three days of the experiment, the graft still preserved some parts of the cancellous tissue below the cortex. The trabeculate present was original from the grafted bone and had no connection with the recipient bed. Nevertheless, this interface was not filled by fibrosis or inflammation. In the first week after grafting, inflammation due to the surgical procedures, predominantly neutrophílic, was restricted to the soft tissues above the graft, with no infiltration on bone tissue. With the succession of days after the procedure, collagen deposits began to appear around the graft. However, the area between the graft and the recipient bed was exempt from fibrosis.

The integration between the graft and the mandible was clear from the 14th day, when bone trabecules settled at the space below the cortex of the graft and connected it to the jaw. Such event was more abundant where the perforations were made at the recipient bed. Bone neoformation at the lateral portions of the graft increased the adhesion of the bone fragment to

jaw cortex. This phenomenon was clearly demonstrated on the 56th day of the experiment, when the graft trabeculate was thinner, due to the resorption process.

These events were present in every rabbit regardless of graft nature and PRP use.

3.3 Morphometric analysis

The total area of bone matrix at autologous and allogeneic grafts, in the presence and absence of PRP, is distributed in Table 1. Increase of the bone matrix was observed in both grafts studied in the presence of PRP. Differences were statically significant at the 14th and 28th days for the autologous graft and in the 7th day for allogeneic one. Grafts presented progressive lower indexes of bone matrix starting from the 28th day. PRP interfered in the thickness of the cortex, identified by the increase of the values in almost all of the analyzed subgroups, despite the statistical differences found in the 7th day for the autologous and allogeneic grafts (Table 1).

Table 1 – Total bone graft matrix and cortical thickness according to different grafts nature, PRP addition and periods of observation.

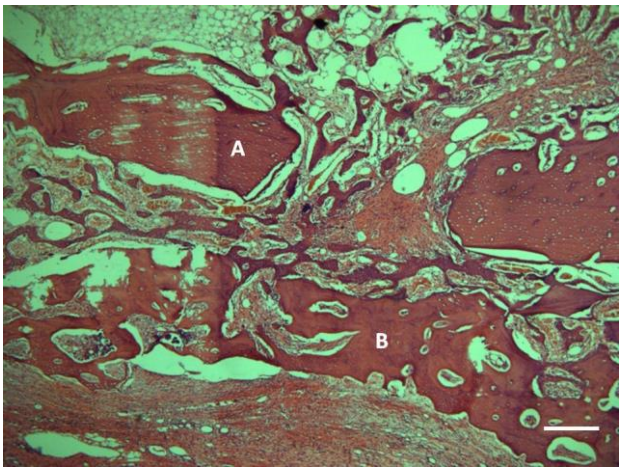
Period	***Total bone graft matrix (mm^2)***				***Cortical graft thickness (mm^2)***			
	Autogenous graft		*Alogenous graft*		*Autogenous graft*		*Alogenous graft*	
	PRP Media ±SD	*No PRP Media ±SD*	*PRP Media ±SD*	*No PRP Media ±SD*	*PRP Media ±SD*	*No PRP Media ±SD*	*PRP Media ±SD*	*No PRP Media ±SD*
03 days	4.81±1.81	3.30±0.93	4.11±1.29[c]	3.66±1.77	0.41±0.18	0.47±0.15	0.56±0.14	0.56±0.18[c]
07 days	4.23±1.21	3.74±0.89	4.34±1.60[a]	2.47±0.92[a]	0.60±0.26[a,c]	0.47±0.12[a]	0.64±0.16[a]	0.51±0.13[a,d]
14 days	4.73±1.48[a]	2.95±0.83[a]	4.32±0.92	4.03±1.04	0.42±0.15[b,d]	0.37±0.13	0.51±0.11[b,d]	0.53±0.16[e]
28 days	4.73±1.03[a]	2.86±0.37[a]	5.76±1.08[c]	4.40±1.03[e]	0.43±0.12[b,d]	0.34±0.11[b,c,d]	0.58±0.09[b]	0.51±0.16[b,f]
56 days	3.31±0.72	2.38±0.48	3.82±1.45	2.45±0.83[e,f]	0.34±0.10[c,d]	0.31±0.09[c,d]	0.43±0.12[c,d,e,f]	0.32±0.14[c,d,e,f]

Tukey´s test:
[a] $p<0.05$ for the differences between PRP use or not for each graft.
[b] $p<0.05$ for the differences between graft natures at each side.
[c] $p<0.05$ for the difference between the 3rd days and the others at each side of mandible and each graft.
[d] $p<0.05$ for the difference between the 7th days and the others at each side of mandible and each graft.
[e] $p<0.05$ for the difference between the 14rd days and the others at each side of mandible and each graft.
[f] $p<0.05$ for the difference between the 14rd days and the others at each side of mandible and each graft.

Source: Authors.

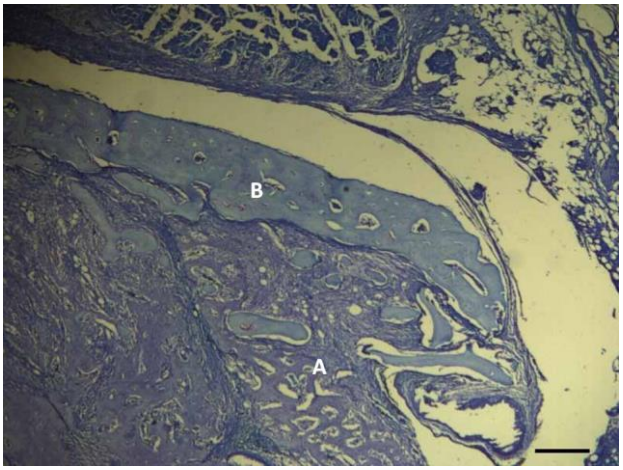
The area of the neoformed bone matrix, in the studied groups, showed differences for each graft type in some subgroups when PRP were added at the moment of surgery according to Table 2. The presence of PRP allowed for a larger formation of the bone matrix, as in the allogeneic and in the autologous group (Figures 1 and 2).

Figure 1 – Interface between the bone graft (A) and the recipient bed (B). Allogeneic graft without PRP, 14th day. HE 40X, Scale = 0.3mm.



Source: Authors.

Figure 2 – Bone neoformation (A) below the graft córtex (A). Autologous bone with PRP, 14 th day. Toluidine blue. 40X, Scale=0.3mm.



Source: Authors.

Collagen matrix density in the soft tissues above different grafts are distributed in Table 2. In bone grafts of the same nature, the collagen density in addition of PRP were similar or superior to the side without PRP (Figure 3). No statistical significances were achieved.

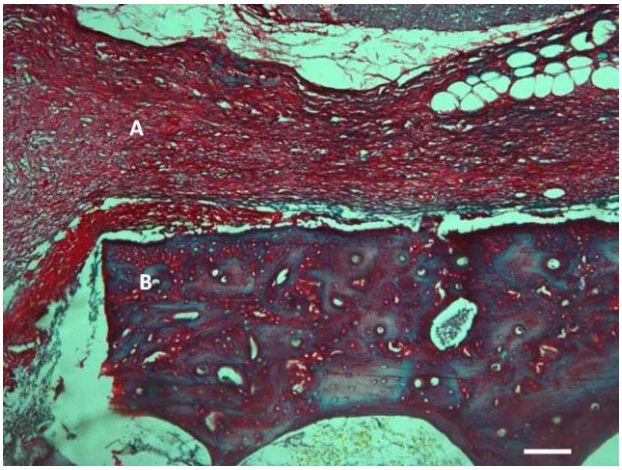
Table 2 – New formed bone matrix secreted at the graft region and area of RAM11 according to different grafts nature, PRP addition and periods of observation.

Period	***New formed bone matrix (mm²)***				***RAM11+ cells area X10² (mm²)***			
	Autogenous graft		*Alogenous graft*		*Autogenous graft*		*Alogenous graft*	
	PRP Media±SD	*No PRP Media±SD*	*PRP Media±SD*	*No PRP Media±SD*	*PRP Media±SD*	*No PRP Media±SD*	*PRP Media±SD*	*No PRP Media±SD*
03 days	0.30±0.23	0.14±0.16	0.21±0.08	0.14±0.11	0.11±0.05[b]	0.13±0.06[b]	0.31±0.14[a,b]	0.46±0.24[a,b]
07 days	0.29±0.24	0.31±0.23	0.34±0.17	0.38±0.33	0.14±0.03[b]	0.17±0.06[b]	0.29±0.11[b]	0.32±0.13[b,c]
14 days	1.71±0.56[a,c,d,e]	0.78±0.42[a]	1.39±0.78[c]	0.79±0.30	0.14±0.05[a,b]	0.21±0.10[a,b,c]	0.34±0.12[b]	0.35±0.10[b,c]
28 days	0.88±0.46[b,e,f]	0.48±0.25	2.17±1.24[a,b,c,d,e]	1.12±0.49[a,c,d]	0.09±0.03	0.10±0.03[c,d,e]	0.15±0.14[b,c,d,e]	0.18±0.10[b,c,d,e]
56 days	0.86±0.55[e,f]	0.75±0.40	0.94±0.41[b,c,f]	0.68±0.35	0.03±0.03[b,c,f]	0.05±0.03[b,c,d,e]	0.10±0.05[b,c,d,e]	0.12±0.07[b,c,d,e,f]

Tukey´s test:
[a] p<0.05 for the differences between PRP use or not for each graft.
[b] p<0.05 for the differences between graft natures at each side.
[c] p<0.05 for the difference between the 3rd days and the others at each side of mandible and each graft.
[d] p<0.05 for the difference between the 7th days and the others at each side of mandible and each graft.
[e] p<0.05 for the difference between the 14rd days and the others at each side of mandible and each graft.
[f] p<0.05 for the difference between the 14rd days and the others at each side of mandible and each graft.

Source: Authors.

Figure 3 – Fibrosis (A) around the bone grafted (B). Allogeneic graft with PRP, 7th day. Picro-sirius, 100X. Scale=0.1mm.



Source: Authors.

The immunohystochemical analysis of macrophage RAM11+ areas is represented on table 3. During the investigation, smaller values of these cells were verified in the autologous graft in relation to allogeneic, representing larger antigenicity in the last group during the initial periods. A tendency to decrease the macrophages population was observed after the 14th day. In the group with the same graft type, smaller macrophage areas were measured when PRP were added (figure 4). In all samples evaluated, no CD79αcγ positive cells were found. Rare CD3+ cells were observed in some rabbits, however they were distant from the graft and therefore were not considered. In the sections used as positive control, the demarcation of those antibodies was in accordance with the expected position for B- and T-lymphocytes.

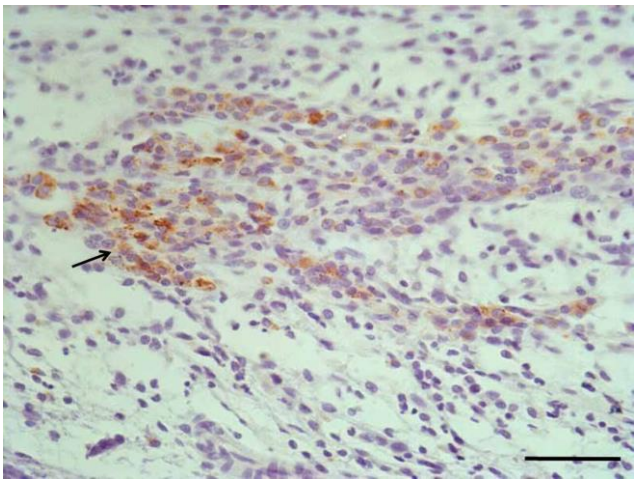
Table 3 – Area of collage according to different grafts nature, PRP addition and periods of observation.

Period	***Collagen density(mm²/0.25mm²)***			
	Autogenous graft		*Alogenous graft*	
	PRP Media±SD	*No PRP Media±SD*	*PRP Media±SD*	*No PRP Media±SD*
03 days	0.08±0.03	0.08±0.04	0.08±0.02	0.07±0.03
07 days	0.11±0.03	0.09±0.04[b]	0.13±0.03[c]	0.15±0.07[b,c]
14 days	0.11±0.02	0.11±0.02	0.10±0.04	0.09±0.03
28 days	0.12±0.03	0.10±0.03	0.09±0.03	0.09±0.03
56 days	0.09±0.04	0.08±0.02	0.08±0.02[d]	0.08±0.03[d]

Tukey´s test: [b] $p<0.05$ for the differences between graft natures at each side.
[c] $p<0.05$ for the difference between the 3rd days and the others at each side of mandible and each graft.
[d] $p<0.05$ for the difference between the 7th days and the others at each side of mandible and each graft.

Source: Authors.

Figure 4 – Macrophages RAM11 positive (arrow) at the soft tissue above the graft. Allogeneic bone with PRP, 3rd day. 400X, Scale = 0.1mm.



Source: Authors.

Table 4 shows the number of osteoclasts and the measure of its area in accordance with graft type, presence of PRP and days of observation. The analysis of these cells had little statistical significance due to their small number in the environment of the studied grafts (Figure 5).

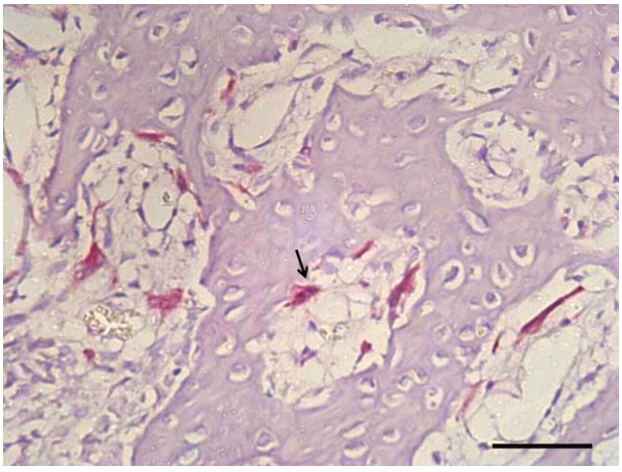
Table 4 – Median, media e standard deviation for osteoclast numbers and area according to different grafts nature, PRP addition and periods of observation.

Observação	*Category*	*Osteoclastos count*		*Osteoclastos area (X10^{-3})*	
		Median	*media±SD*	*Median*	*media±SD*
***Graft nature*[1]**	*Autogenous*	0.5	20±33.57	0.15	2.83±4.85
	Alogenous	7	29±56.26	1.20	3.64±6.23
***Side*[2]**	*with PRP*	5	23±37	0.85	2.99±4.80
	without PRP	3	27±55	0.40	3.47±6.31
***Days after graffting*[3]**	*3*	0	0	0	0
	7	3	8±10	0.45	1.12±0.08
	14	62	76±71	8.00	9.40±7.80
	28	24	36±40	3.10	5.13±5.55
	56	0	4±9	0	0.52±1.05

1. Mann-Whitney´s test. Osteoclast count p<0.195; Osteoclat area p<0.173.
2. Mann-Whitney´s test. Osteoclast count p<0.950; Osteoclat area p<0.547.
3. Kruskal-Wallis´ test. Osteoclast count p<0.0001; Osteoclat area p<0.0001.

Source: Authors.

Figure 5 – TRAP-positive cels (arrow) inside the graft. Allogeneic graft without PRP, 14th day. TRAP, 400X, Scale = 0.1mm.



Source: Authors.

In all variables, a superiority on the behavior allogeneic graft was observed, especially in the final periods of the experiment.

4. Discussion

In spite of that, the literature lacks works that deal with the regeneration process between the recipient bed and the grafted boné (Roldan, 2004). Therefore, this work assumes singular relevance, since the events in the micro environment of the healing process, from the beginning of graft repair until its absorption, were investigated in the presence of modifier variables.

In relation to the bone graft matrix measurement, a similarity was found among allogeneic and autologous grafts even when the presence of PRP was considered. In the analysis of the autologous grafts, there was a decrease on the values of bone matrix in the final periods of the experiment, which is a result of the absorption process.

This is a predictable event when a segment of bone at the alveolar ridge of the maxillaries is not submitted to physiological stress. In other experiments, the absorption of the autologous bone was more significant, even in the presence of PRP (Ohya, et al. 2005). This growth factors concentrate contributed, in the present work, to a larger amount of the original autologous graft after absorption process initiate (Aghaloo, et al. 2002).

However, allogeneic grafts preserved a larger area of bone matrix in the final days, which suggests that the theses grafts are more resistant to the absorption process. Lucarelli, et al. (2005) also found favorable results with the use of allogeneic grafts. Studying metatarsal reconstruction, these authors evidenced new bone formation with better vascularization if allogeneic bone grafts were associated to PRP. However, the accomplishment of a descriptive analysis, the addition of collagen and steam cells in the group treated with PRP have perhaps influenced their results.

In relation to the thickness of the cortical, larger values in allogeneic grafts were noticed. Hence, it was possible to confirm the superiority of the cortex of the allogeneic bone grafts. Other authors had already established that the cortex of the frozen bone is more resistant to the absorption than the cortex of a fresh one (Kluger, et al. 2003, Tshamala, et al. 1999). This phenomenon occurs due to a reduced osteoclast adhesion to tissue after bone freezing since low temperatures promote integrins denaturation.

PRP represented a protection feature for cortex thickness of the grafted fragments, although it showed statistical significance only on the 7th day. Besides that, the cortex portion of the grafts, independent of its nature, was more resistant to the absorption process when the bone had been grafted with PRP. In the literature, the thickness of the cortex was only obtained around titanium implants when PRP was used in association with steam cell (Yamada, et al. 2004).

Bone neoformation below the cortex and at the periphery of the grafts took place in later periods on allogeneic bone. However, such phenomenon was observed in a more homogeneous and significant way in relation to the autologous grafts. Along the experiment, the findings contradict the arguments of Kluger et al (2003) who defend that, although more resistant to absorption, frozen bone is less osteogenic. Moreau et al (2000) proposed that freezing and chemical treatment of the bone marrow increase graft osteogenic potential, due to alterations in cholesterol composition and other fatty acids present in adipocytes, which increases the osteogenic potential of the graft.

The study of collagen matrix around of the grafted fragments did not show significant differences, even regarding the nature of the grafts or the use of PRP. In general, the synthesis of collagen was discreetly higher in the presence of PRP. Several authors reported that fibrosis induced by PRP has clinical significance. The repair of the soft tissues occurred in a safer and faster manner due to the presence of this growth factor concentrate (Carneiro Mde, et al. 2013, Kimura, et al. 2005).

In relation to macrophage chemotaxis, significant differences were found between the two graft natures. The largest amount of macrophages was detected around the allogeneic bone, regardless of the fact that bones of such nature are less absorbed. Macrophage is recognized as essential in the mechanism of rejection of this type of graft, due to its role as antigens presenting (Carpenter, & Tomaszewski, 1998, Tshamala, et al. 1999). As a result, allogeneic bone, even with the treatment against antigenicity, can still present epitopes that will be phagocyted by macrophages. (Dohan, et al. 2006) Nevertheless, as well as platelets, macrophages also release growth factors that are important to stimulate the osteogenesis (Anitua, 1999). They can act on pre-osteoblasts, stimulating its proliferation. After its differentiation, these bone cells amplify that process through GM-CSF synthesis in a positive feedback with macrophages (Nordstrom, et al. 1999). According to several authors, such cascade of events allows for a better repair and better incorporation of bone grafts (Lari, et al. 2007, Nordstrom, et al. 1999).

The interface of the graft with the recipient bed, in all observational stages, was always exempt from lymphocytes at hematoxilin and eosin staining. This finding was confirmed by the absence of B- or T-lymphocyte markers, which consolidate the supposition that frozen allogeneic bone is poorly immunogenic (Nordstrom, et al. 1999, Tshamala, et al. 1999).

In this study, it cannot be stated that the osteoclasts had any fundamental role in bone absorption. Osteoclasts were

more present in the days of larger osteogenicity, what is in accordance with the biological relationships established between these cells and osteoblastos (Asagiri, & Takayanagi, 2007). Small decrease of these cells was observed in the final periods of the experiment. It is believed, therefore, that the absorption process of grafts might have been governed by other mechanisms.

5. Conclusion

Thus, the indication of allogeneic grafts for onlay maxillaries reconstruction is reliable. Freezing was a practical and effective method for the decrease of the immunogenicity. The use of allogeneic frozen bone eliminates a second surgical bed. This type of graft has high osteogenic potential and high resistance to absorption. The mechanisms responsible for the absorption of onlay graft bone are still ignored and this work rejects the hypothesis that PRP is an important variable that could improve healing.

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