Brazilian Dental Science
Ciência Odontológica Brasileira
unesp
UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"
Instituto de Ciência e Tecnologia
Campus de São José dos Campos

ORIGINAL ARTICLE

doi: 10.14295/bds.2014.v17i4.998

Histomorphometric, radiographic and biomechanical analyses of platelet rich plasma associated with autogenous bone graft

Efeito do plasma rico em plaquetas associado ao enxerto autógeno na tíbia de coelhos: estudo histomorfométrico, radiográfico e biomecânico

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ABSTRACT

Objective: The aim of this study was to evaluate the effectiveness of platelet rich plasma with and without autogenous bone graft in the bone repair of surgical defects in rabbit tibias. **Material and Methods:** In this research, 25 adult male rabbits were used. Two defects have been performed in each tibia, divided into four groups: control (C = defect naturally left to heal by clot formation), autogenous (A = bone defect + autogenous graft), PRP (PRP = bone defect + PRP) and autogenous + PRP (PRPA = bone defect + autogenous graft + PRP). All the defects were covered with a dPTFE membrane. Five other animals were sacrificed at 15, 30, and 60-day postoperatively. The pieces containing the defects were processed for histological and histomorphometric analysis. Other five animals were sacrificed after 30 and 60 days and submitted to biomechanical analysis, and all the specimens were sent to the radiographic evaluation of optical density. **Results:** The biomechanical, radiographic, and histomorphometric results showed larger resistance, optical density, and improving bone formation in the groups A and PRPA when compared with the groups C and PRP. **Conclusion:** This study showed there was not an improvement in the radiographic, mechanical, and bone formation parameters when PRP was used individually or associated to the autogenous bone graft.

KEYWORDS

Bone grafting; Guided tissue regeneration; Platelet rich plasma; Polytetrafluorethylene; Membranes.

RESUMO

Objetivo: O propósito deste trabalho foi avaliar o efeito do plasma rico em plaquetas associados ou não ao enxerto ósseo autógeno no processo de reparação óssea em defeitos cirúrgicos confeccionados na tíbia de coelhos. **Material e Métodos:** Nesta pesquisa foram utilizados 25 coelhos adultos, nos quais foram realizados 2 defeitos em cada tíbia, divididos nos seguintes grupos de acordo com o tratamento: controle (C - defeito preenchido somente por coágulo sangüíneo), autógeno (A - defeito + enxerto), PRP (PRP = defeito + PRP) e autógeno + PRP (PRPA - defeito + enxerto + PRP). Todos os defeitos foram recobertos com uma barreira de PTFE e decorridos 15, 30 e 60 dias, 5 animais foram sacrificados por período, sendo as peças contendo os defeitos processadas para análises histológica e histomorfométrica. Outros 5 animais foram sacrificados aos 30 e 60 dias e submetidos à análise das propriedades biomecânicas e todos os espécimes foram submetidos ao exame radiográfico para análise da densidade óptica. **Resutados:** Os resultados biomecânicos, radiográficos e histomorfométricos mostraram maior resistência, densidade óptica e maior formação óssea nos grupos A e PRPA quando comparados com os grupos C e PRP. **Conclusão:** Os resultados obtidos possibilitaram concluir que não houve uma melhora nos parâmetros radiográficos, mecânicos e na neoformação óssea quando o PRP foi usado isoladamente ou associado ao enxerto ósseo autógeno.

PALAVRAS-CHAVE

Plasma rico em plaquetas; Regeneração óssea; Enxerto ósseo; Membranas; Politetrafluoretileno.

INTRODUCTION

Reconstructive surgical techniques are conditions for aesthetic and rehabilitation, especially for correcting bone loss because of trauma, cystic lesions, infection, tumors or resorption of alveolar bone. Autogenous bone grafts from intraoral and extraoral sources are considered as the gold standard and used to regenerate bone defects in the craniofacial region. Many studies have sought quantitative and qualitative improvement of the bone grafts results, and guided bone regeneration (GBR) approach has positive benefits in this aspect [1,2]. The repairing of bone grafts depends on several factors such as quality of donor tissue, vascularization of the receptor area and graft immobilization.

Growth factors, produced by cells such as fibroblasts, osteoblasts, chondroblasts and other mesenchyme cell lines were identified, isolated, and tested on their ability to start bone growth [3]. Platelets contain some of these growth factors and are also rich in cell adhesion molecules [4]. The strategy was to use the platelet rich plasma (PRP) with grafts, suggested by increasing bone productivity and quality of regenerated bone, starting the proliferation, migration and cell differentiation, followed by osteoid and later mineralization.

In osseointegration of endosseous implants, migration and cell differentiation, formation of bone and turnover is dependent on platelets and blood clot. Thus, the PRP can be used to increase the osseointegration in patients for whom this may be less predictable, as elderly and individuals with osteoporosis, diabetes or other conditions in which bone regeneration could be compromised [5]. It is an autogenous material and provides no possibility of cross-transmission of illness or immune reactions. The use of PRP improves handling the particulate graft material and insertion into the surgical cavity, allowing to attach a denser and more effective graft and in preserving the defect local area, and resulting bone regeneration [6-8].

Despite the scientific evidence that platelets and the PRP work in the first phase of biochemistry and early bone regeneration, there is still no consensus on the benefits of its use associated with graft material [9]. The aim of this study was to evaluate the effectiveness of PRP, with or without autogenous bone graft, using guided bone regeneration strategy in repair of bone surgical defects created in the tibia of rabbit. This study was performed by radiographic, microscopic and biomechanical analysis.

MATERIAL AND METHOD

Animals and experimental groups

This study has done according to the Brazilian Animal Experimentation College (COBEA) guidelines and approved by the Research Ethic Committee of the São José dos Campos Dental School – UNESP (nº 026/2005-PA/CEP). Twenty five rabbits (adult, *New Zealand*, male, 3.5 kg average weight) were kept in individual cages with a healthy food and water ad *libitum*. Each animal had four bone defects divided into four groups: Control (C = bone defect), Autogenous (A = bone defect + autogenous graft), PRP (PRP = bone defect + PRP), and PRP + Autogenous (PRPA = bone defect + PRP + autogenous graft).

PRP extraction

Eighteen milliliters of blood were harvested by heart puncture and separated into four Vacutainer collection tubes (Becton-Dickinson, Belliver Industrial Estate, Plymouth, UK) containing 0.5 mL buffered sodium citrate as an anticoagulant. The blood samples were centrifuged at 1200 rpm for 10 min at room temperature for separation of plasma and red blood cells. The plasma phase was collected for a second centrifuge set at 1200 rpm for 10 min at room temperature to obtain two phases: PRP (lower phase) and PPP (platelet poor plasma) at upper phase. PRP phase was collected and homogenized with 10% calcium chloride (Terapêutica, São José dos Campos, SP, Brazil) at 8:1 proportion. Calcium chloride promotes

reverting sodium citrate chelation, and this response allows use of PRP in gel form.

Platelet count

Platelet count was made in whole blood, and PRP by cubic millimeter, diluting 20 μL of whole blood or PRP in 2 mL 1% ammonium oxalate and homogenized. A Neubauer chamber was used for the platelet count in a 400X optic microscope magnification. Considering the dilution, the result of the count was multiplied by 10^3 and obtained an absolute value of the platelet number by mm^3.

Surgical proceedings

The antibiotic Pentabiótico (0.1 mL/kg) (Fort Dodge Saúde Animal Ltda, Campinas, SP, Brazil) and anti-inflammatory Voltaren 75 mg (0.6 mL/kg) injectable solution (Parker-Davis, São Paulo, SP, Brazil) were administrated one hour before the surgical proceedings. The animals were sedated with analgesic-sedative and muscle-relaxing (1 mL/kg) Rompum (2% watery solution of 2-(2,6-xilidine)-5,6-dihydro-4H-1,3 thiazin, Bayer, Brazil) five minutes before administration of general anesthetic (0.5 mL/ kg) Dopalen (ketamine hydrochloride, Agribrands do Brasil Ltda, Brazil).

Animals shaved, and asepsis made with 0.2% chlorhexidine solution (Terapêutica, São José dos Campos, SP, Brazil). Two tibias unicortical defects were made by an electric surgical motor Asseptico (AEU-707-MGF Asseptico Inc., USA) using a 5 mm trephine drill, 1/16 reduction at 1500 rpm, and sterile 0.9% sodium chloride continuous irrigation. Bone fragments from bone defects were particulate and used as autogenous graft material.

One bone defect was filled by the clot and other by autogenous graft, denominated control (C) and autogenous (A) groups respectively in each animal right tibia. Left tibia defects were filled by PRP (PRP) and autogenous bone graft associated with PRP (PRPA). All bone defects were covered by dPTFE membrane to prevent the nonosteogenic tissue invasion. Deep tissue layers were sutured with Vicryl (Ethicon, Johnson & Johnson, USA) and silk 4-0 (Ethicon, Johnson & Johnson, USA) for superficial tissue layers.

Sacrifice periods

Five animals of each period sacrificed after 15, 30, and 60 days by anesthetic overdose. Tibias containing bone defects harvested and fixed in 10% formalin (Merck KGaA, Darmstadt, Germany) for 72 h. The bone defects were radiographed and processed for microscopic and histomorphometric analysis.

Five animals sacrificed after 30 and 60 days after surgical proceedings for biomechanical analysis. Blocks containing bone defects removed and frozen in Ringer solution for radiograph and biomechanical tests.

Optical density analysis

The Visualix Gx-S-HDI system (Gendex Dental System, Dentsply International, Chicago IL, USA) was used for obtaining radiographic images regulated at 65 KVp and 7 mA. The bone defect blocks placed on sensor equipment at 40 cm of distance for 0.02 s x-ray exposure. A blind study was conducted for obtaining the amount of density in each region using Image Tool version 3.0 (University of Texas HSC at San Antonio Dental School, TX, USA) image analysis.

Histological and histomorphometric analysis

The blocks were demineralized by 10% solution of EDTA Titriplex III (Merck KgaA, Darmastadt, Germany) and processed in paraffin. Histological sections with 6 μm thickness was stained with hematoxylin-eosin and Mallory trichrome. Histomorphometric study was made by the stereologic method to find out quantitative three-dimensional anatomical parameters.

Three slices, randomly used of ten semiserialized histological slices from longitudinal cut through the center of the bone defect of each animal, were evaluated by Axioscope 40 light microscope (Carl Zeiss, Germany) and Axiovision program (Carl Zeiss Vision Imaging, Carl Zeiss, Germany). Five

439.28 x 329.12 μm2 area image fields were capturated by AxioCam MRc5 (Carl Zeiss, Germany) to determine the density of bone. In two histological image were evaluated the number of osteoblasts available in new bone, in the border of the defect.

Biomechanical properties

An electromechanical tool for tensile and compression tests model MEM 10.000 used to evaluate the compression strength of new formed bone. The speed of the load cell (3.1 mm diameter cylindrical 20 kgf) application during compression tests was constant at 1mm/sec until specimen failure. The tests were made at room temperature, and specimens kept moist in saline solution. The resistance to compression was calculated using following formula:

$$\sigma_c = 9.81 \times 4 \times F_{max}/\pi D2$$

σ_c = Resistance to compression or tension of compression of new formed bone (MPa)

9.81 = Gravitational force (kgf)

F_{max} = Compression maximum load (kgf)

D = Diameter of load cell (3.1 mm)

Statistical analysis

The results of optical densitometry, histomorphometric and biomechanical properties were submitted to ANOVA RM statistical analysis. One assessment was between the groups (period of treatment) and other within the group (treatment performed). The statistical significance level was $p = 0.05$. Furthermore, the results were submitted to Tukey's multiple comparison tests.

RESULTS

Platelet count

Platelet count showed the platelets average of 280 x 10^3/mm^3 (230 x 10^3/mm^3 to 315 x 103/mm^3) in peripheral blood, and average of 1052 x 10^3/mm^3 (848 x 10^3/mm^3 to 1260 x 10^3/mm^3) in ready PRP.

Optical density analysis

Most noted radiographic findings in control and experimental groups were analyzed together in each period of the considered trials.

Fifteen days - Radiographic aspects were similar between C/PRP and A/PRPA groups. It was noted radiolucent area of the bone defect showing detailed and defined limits in C and PRP groups. The A and PRPA groups showed radiolucent image size of the bone defects displaying strict limits but undefined in some areas, and radiopaque images in the bone defect equivalent to bone graft particles.

Thirty days - The bone defect topographies were visualized, whereas the limits were precise and discreetly undefined in some defects of C and PRP groups. In A and PRPA groups bone defects limits were identical, irregular, showing loss of sharpness in some regions, but defect topography could be viewed. The reduced radiographic density of bone defect walls and radiopaque images of the bone particles presented more consistent in the center matching to bone graft.

Sixty days - Radiographic images of the defect and the wall limits were still visible in C and PRP groups, although there was evidence of a density increase in the contour of the bone defect. The topography of the bone defect was barely visible with loss of sharpness in the contour of the defect walls in A and PRPA groups. There were regions of a defect showing similar radiographic density when compared to density of normal bone tissue, although there were radiopacity differences in regions in the same bone defect. The images of bone graft particles had become more homogeneous, compared to the previous periods. Furthermore, centripetal bone growth was observed because of density variation between the peripheral and central radiographic areas.

The averages of density in the bone defect showed more optical density in A and PRPA groups when compared to C and PRP groups. There was also a reduction in optical density in all groups in all periods. According to data of ANOVA analysis, there was a significant

statistical difference between optical densities of studied groups and in the experimental periods. Tukey's multiple comparison test showed statistically significant differences only for the period of sixty days and revealed significance between A and PRPA to C and PRP in different periods (Figure 1).

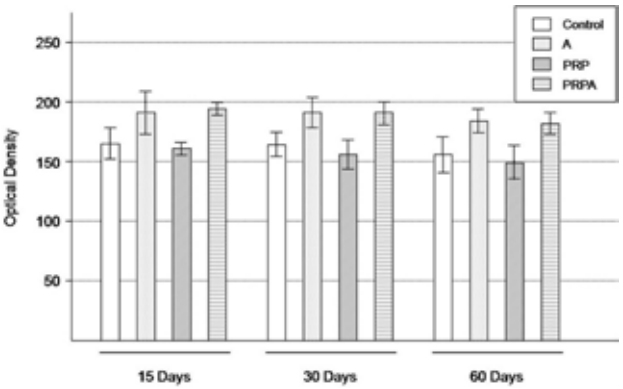

Figure 1 - Graphic representation of the optical density averages evolution of C, A, PRP and PRPA groups in 15, 30 and 60 days.

Histological and histomorphometric analysis

Frequent histological findings in the species were analyzed together within each period considered in the experiment.

Fifteen days - In the defects of C and PRP groups, the limits showed new formed bone trabeculae and the central area filled by osteogenic connective tissue. It was noted bone turnover in the limits of defects, characterized by osteoclastic resorption and bone matrix deposition by osteoblasts, and necrotic areas with empty osteoplast. Bone trabeculae were immature, thin, basophilic with linear arrange and extending to the central portion presenting a bulky and large number of osteoblasts (Figures 2a and 2b).

The osteogenic connective tissue was highly cellular and vascularized, comprised of osteoprogenitor cells and collagen fibers arranged in parallel with each other. A fibrous connective tissue was observed on the surface of the defect, an area previously occupied by PTFE barrier. New formed blood vessels and edema were more frequently noted in PRP when compared to C group (Figure 2c). Collagen fibers of osteogenic connective tissue were more delicate in PRP group than C (Figure 2d).

The defects were filled by immature thin bone trabeculae, and by osteogenic connective tissue highly cellular and vascularized in A and PRPA groups (Figures 3a and 3b). The trabeculae showed a linear arrangement and extended

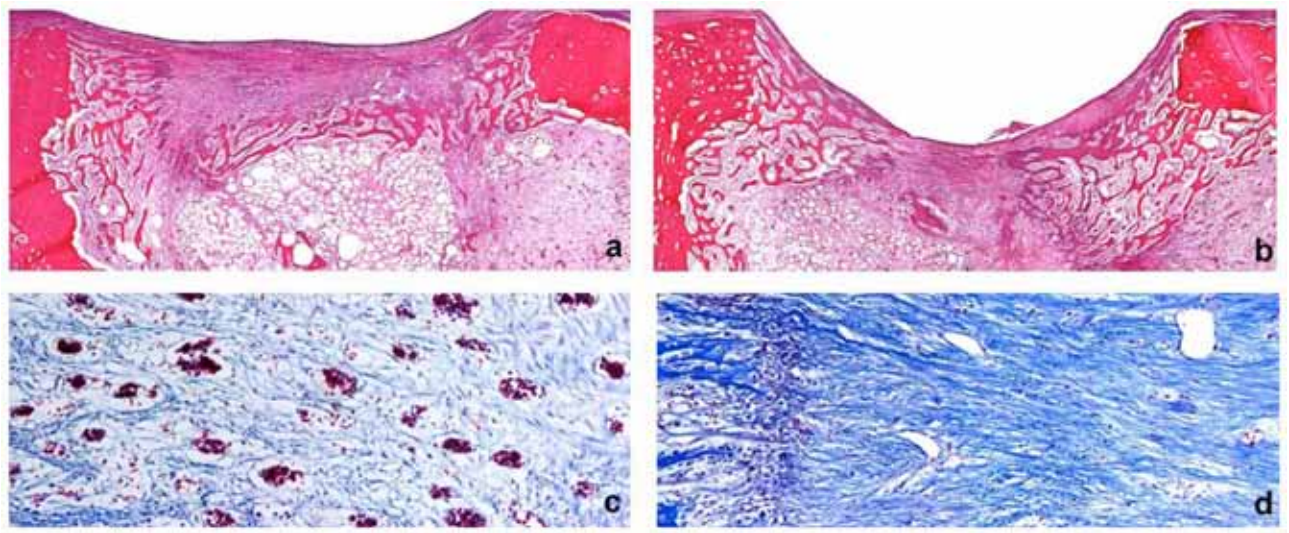

Figure 2 - Regions of bone defects at day 15: a) C group; b) PRP group, Osteogenic connective tissue; c) diffuse aspect and well vascularized in PRP group; d) dense aspect with intense osteoblastic activity and formation of bone trabeculae in C group. HE and Mallory trichrome, 50 e 100 x original magnification.

to the central portion. Graft particles were found in a central region of a defect showing integration with osteogenic tissue and newly formed trabeculae. Some particles showed empty osteoplast. Osteoclastic resorption was more severe in PRPA group than A in the graft particles (Figures 3c and 3d). Bone turnover and necrotic areas were found in the defect edges. In addition, more vascularization and sometimes fibrin networks and hemorrhagic areas were seen in PRPA when compared to A group.

Thirty days - In C and PRP groups, the region of a defect was filled by osteogenic connective tissue (Figures 4a and 4b). It was noted an increase of bone trabeculae, lamellar in the border and immature in the center, when compared to the fifteen-day group. In these immature regions, it was noted less bulky and flat osteoblasts, areas of bone turnover and presence of osteoclasts and basophilic lines. There was a thin bone layer over on the center surface of a defect in most of the histological slices, without integration with defect borders. Under this thin bone layer, in a central and deeper part, it was noted a great amount of well-organized osteogenic connective tissue with immature bone trabeculae with discreet and diffuse infiltration of mononuclear inflammatory cells. New formed bone marrow was noted in the edges of a defect in PRP group, the vascularization was more intense, but the bone marrow was less developed when compared to C group.

There were no significant differences between A and PRPA groups. Both showed likenesses, such as new formed bone and osteogenic connective tissue. Fat and hematopoietic cells sparsely noted in marrow spaces close the bone defects. Resorption of graft particles and active bone formation was also noted. New formed bone with the trabecular features showed to a linear arrangement with several osteoblasts and osteocytes. The first signs of bone turnover and Harversian systems development could be seen surrounded by immature bone marrow. Interconnections

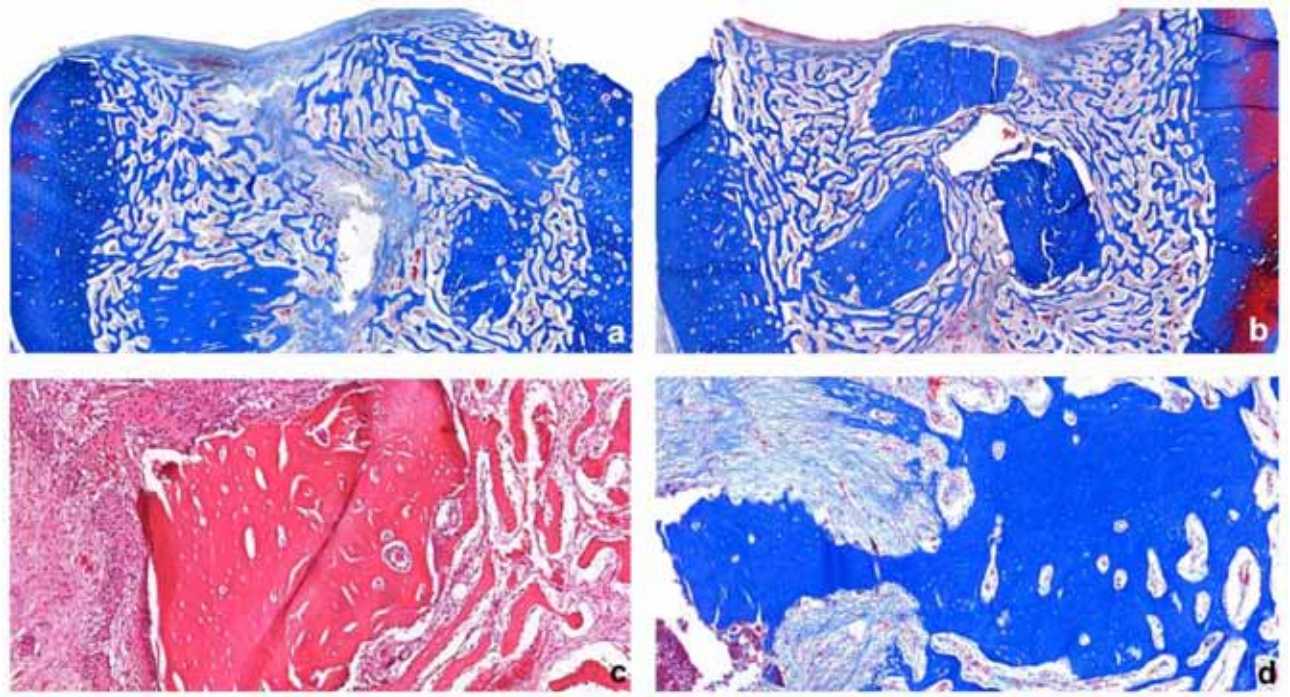

Figure 3 - Surgical defect area at day 15: a) A group; b) PRPA group; c) graft particles surrounded by osteogenic connective tissue and by immature bone trabeculae in A group; d) intense osteoclastic activity and reabsorption of graft particle in PRPA group. HE and Mallory trichrome, 50 e 100 x original magnification.

between thick bone trabeculae were noted at the edges of the defect and extending to the central portion of a linear form. In this region, there was a presence of osteogenic connective tissue showing more substantial osteogenic cells and parallel collagen fibers with small fibroblasts. The bone trabeculae were immature, thin, with large marrow spaces in the central portion. It sometimes was difficult to determine the defect borders, because bone turnover regions are present in these areas (Figures 4c and 4d).

Sixty days - The bone defects were filled by mature and immature bone tissue, and by osteogenic connective tissue in C and PRP groups. The edges of a defect showed unclear and the thickness similar or sometimes thinner than the thickness of the original cortical. A fibrous connective tissue was noted over the bone defect surface, delimiting the space previously occupied by the barrier. The marrow spaces localized on the defect border was filed by marrow tissue with similar characteristics to normal bone marrow. In central portions, these spaces were filled by loose connective tissue. Areas of bone turnover and Haversian systems formations were noted in all layers of bone (Figures 5a and 5b).

The groups A and PRPA showed new formed bone and almost complete closure of defects. Bone tissue was mainly mature, presenting a few nonvital residual particles, which were surrounded by new formed bone tissue and rare osteoclasts. There were mature bone trabeculae with bone marrow and turnover evidence in recent formed bone at the edge of the defect. It was also noted bone formation in marrow bone, possibly by bone graft, which had been refurbished and incorporated by bone tissue. Inside the tibia marrow channel sometimes some small graft particles were noted surrounded by connective tissue (Figures 5c and 5d).

The purpose of the histomorphometric study was to evaluate the density of volume of the new formed bone and display the number of osteocytes in defects of different groups (Figure 6). The average of bone volume densities showed that A and PRPA groups revealed more formation of bone tissue when compared to C and PRP groups. According to the data, on the analysis of variance (ANOVA), there was statistically significant difference between the densities of bone volume in the various groups, and periods studied ($p < 0.05$).

Tukey's comparison test revealed a statistically significant difference between groups A and PRPA, and C and PRP. This test

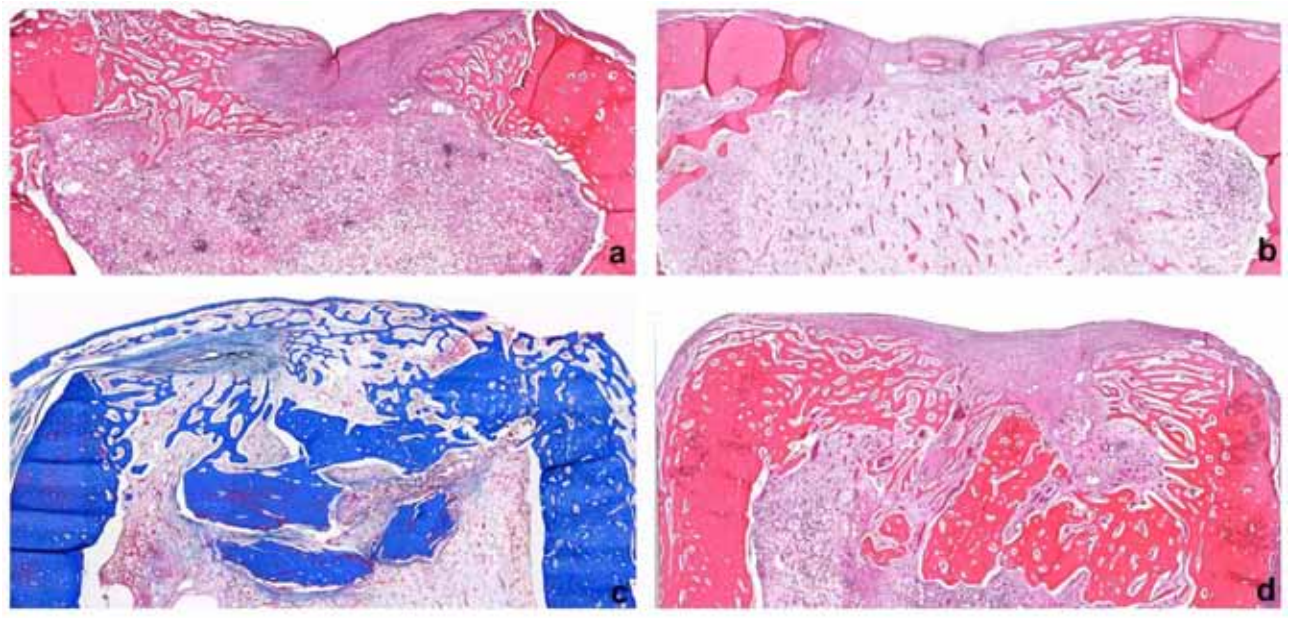

Figure 4 - Bone defect regions at day 30 (arrows): a) C group; b) PRP group; c) A group; d) PRPA group. HE and Mallory trichrome staining 50x original magnification.

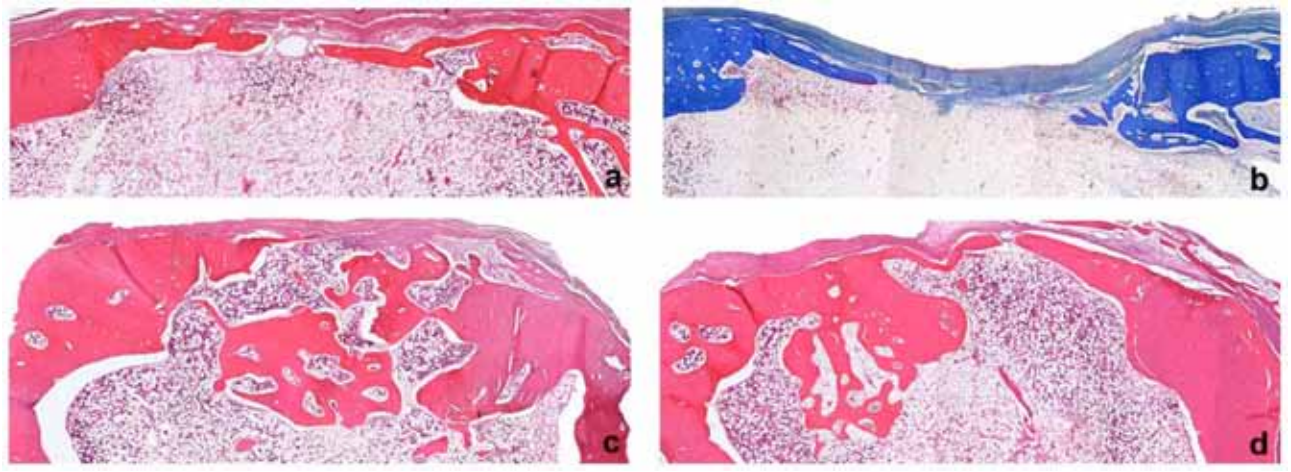

Figure 5 - Bone defect regions at day 60: a) C group, b) PRP, c) A group, d) PRPA group. HE and Mallory trichrome staining. 50x original magnification.

also showed a statistically significant difference between all periods. Figure 7 represents the evolution of density average of bone volume within each group examined over the periods. The graph shows uniformity in progressing new formed bone quality average during in the examined periods.

Biomechanical properties

It analyzes of the results expressed in Figure 8, showed the A and PRPA groups had greater resistance to compression when compared to C and PRP groups. According to results analyzed by ANOVA test, there was a statistically significant difference ($p < 0.05$) between compression forces of various studied groups. There was no relevant variation in the different periods. Tukey's test revealed there was a statistically significant difference between groups C and PRP and between A and PRPA groups.

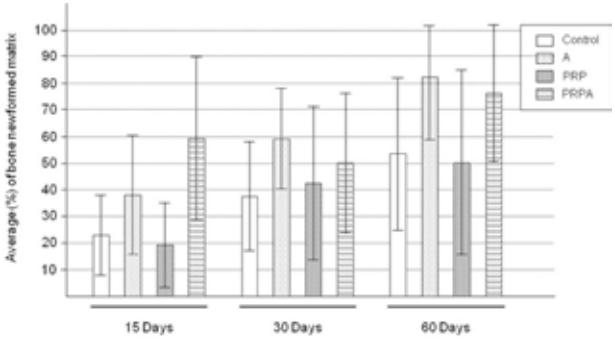

Figure 7 - Graphic representation of new formed bone matrix volume density averages evolution at days 15, 30 and 60 in C, A, PRP and PRPA groups.

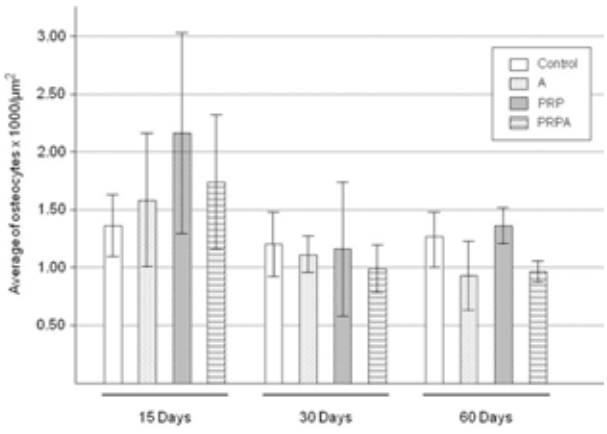

Figure 6 - Representation of osteocytes averages per area (103 µm²) of new formed bone matrix in C, A, PRP and PRPA groups at days 15, 30 and 60.

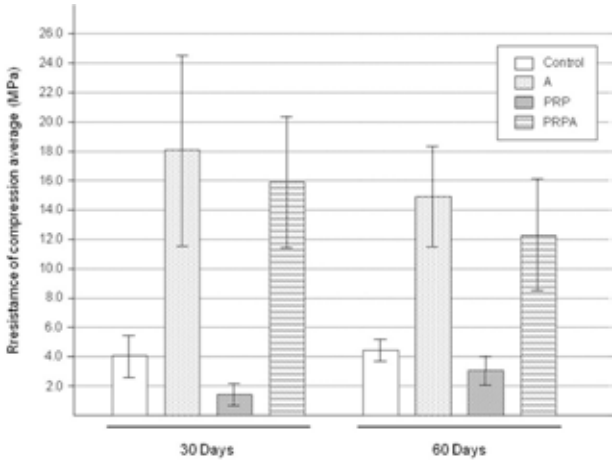

Figure 8 - Graphic representation of compression strength averages applied at specimen defects in C, A, PRP and PRPA groups at days 30 and 60.

DISCUSSION

With the recent advances in reconstructing the bone defects, osseointegration has been directed to increase and improve of the intrinsic cellular response, and application of topical substances has drawn attention. Based on autologous blood, the PRP contains growth factors with various actions, such as stimulation, proliferation, differentiation and chemotaxis of dissimilar cells, speeding up tissue repair and improving bone density [3,10].

Platelets provide significant quantities of growth factors that promote chemotaxis of precursor cells, induce cell differentiation and proliferation, collagen production and the angiogenesis [11-13]. In the present study, it was the existence of higher vascular development in the PRP groups.

PRP platelet concentration had an average increase of 3.8 times compared to whole blood in this research, equivalent to studies that reported a positive effect with the PRP [11]. This PRP extraction process has also shown as reproducible and easy to use. Automated methods of PRP extraction reach concentration ranging over to six times higher [14,15]. Some works described bigger increases in some counts, although there is no correlation between the PRP concentration and new formed bone, while others reported positive effects or even a paradoxical response in higher concentrations [15-20].

The value and benefits of the PRP have been described in various procedures for bone grafts in alveolar resorption, techniques of maxillary sinus lifting or concomitant to the immediate implants installation [5,8,19,21-24]. However, the use of PRP is controversial and earlier studies have shown the PRP association to autologous transplant or bone substitutes increased the percentage of osteogenesis and bone formation [3,5,11,22].

Others studies found beneficial effects only in the early stages of repair. Further, results of some searches did not reveal significant differences [25] nor showed a lower bone formation and a delay in bone grafts remodeling when the PRP was added [16-18,20,26-29]. This work did not find qualitative or quantitative differences in bone formation when the PRP was associated.

Histomorphometric analysis showed some sites with significantly more bone callus formation. In groups that received graft, the highest density was also because of the presence of no reabsorbed graft particles. However, there was a loss of defect limits noted radiographically in later periods, and images were more homogenous, confirmed by histological findings showing absorption and integration of the graft particles.

The bone turnover in humans and rabbits are physiologically similar, but there are differences in the overall architecture of the cortex. The rabbit cortical bone is lamellar, such as human bone, but includes a smaller number of Haversian channels and osteons, characteristic of the thicker cortical bone of primates. Rabbits are commonly used as an experimental model for assessing bone grafts incorporation and immunogenicity. Thus, changes in mechanical properties offer a correlation of biological, immunological, and biomechanical parameters of the graft in the same animal.

The bone volume is important to the correct planning, and the prediction of graft-supported rehabilitation depends on the new formed tissue quality. The A and PRPA groups had greater resistance because of the presence of autogenous graft and osteogenic particles, osteoinduction and osteoconduction effects. Further, bone callus formation has been associated with the mechanical trauma during the defect production. The PRP has influenced connective tissue growing with the more sensitive aspects, which may also have influenced the results seen above in PRP-associated groups.

Bone breakdown or fracture will not appear if the mechanical loads are within the limit of resistance of the grafted site [30]. On the other

hand, in this work, premature external trauma, as muscle insertions and movements, may affect negatively the repair, reducing the resistance and hindering the total repair of defects. In addition, the pathophysiological process stimulates the bone marrow reconstruction, noted in histological evidence as a great resorption of grafted particles in the medullar channel. The only significant increase in bone formation was in the groups receiving graft. However, PRP did not impact positively on quantity or quality of the new formed bone, despite the presence of a great number of osteogenic cells.

The necessity of cells to the combined PRP is still controversial, and when combined with osteoconductive materials, similar results have been reported over the rate of bone formation [31,32]. If adding PRP would only result in speeding up bone formation in the presence of specific cells (osteoblasts and osteocytes), is a problem that remains. For assessing the PRP effect on the bone formation successfully, the ideal method would be an alternative model of bilateral grafts, with the controlled addition of PRP as the only variable.

The osteoclasts measurement is impossible by histomorphometric methods [28]. However, the increased rate of bone removed suggested a greater number of osteoclasts, which presented many nucleuses and acted in the graft region associated with the PRP. Despite the increased vascularization noted in early periods in PRP groups, there was no difference in the result of bone formation in this experimental model, using endochondral bone. However, when associated with intramembranous graft, similar results were found, showing the low regenerative capacity of the PRP [18,26,27,32,33].

The local PRP activity in bone regeneration could be of benefit to the critical size defects with compromised vascularization [11]. However, there was no adjuvant effect on small defects such as in the present model. In small bones (e.g. parietal or tibia) a 15 mm critical size defect could not be created.

Identification of different tissue constituents and cell types can be better achieved by immunohistochemistry techniques [12]. For avoiding occurring errors and false counts of osteoblasts and osteoclasts, a count of osteocytes has been conducted in this study, so these cells could be identified more precisely, because of their presence within the bone. The cell quantification and its relationship to the area of the new formed showed a greater number of osteocytes by area in the PRP group, followed by PRPA, A and C groups, all in the initial period. Further, there was not noted a large amount of bone in this group, suggesting that a great number of cells were associated with the more immature trabeculae. Similarly, the great number of osteoblasts did not create a demonstration of an increase in the bone rate placed by these cells. Histologically, this measurement can only be done evaluating the distance between the tracks of fluorochromes in specific intervals. Using this method, previous studies did not observe an increase in the bone growth rate, despite the augment in concentrating growth factors produced by the PRP. However, an increased amount of new bone was noted and credited to the enlarged number of osteoblasts working in grafted regions [29].

The osteogenesis was similar in the early period, while at 60 days showed turnover. The great number of cells in the PRP group revealed more immature bone and osteogenic connective tissue quality. These findings do not exclude the possibility of the favorable effect of the PRP in the early stages of bone repair, if any lack in these mechanisms exists.

The clinical correlations of this study, which have been extrapolated to the human model, are the PRP association does not make a greater density of graft trabeculae. Furthermore, the final product is not different from the normal bone volume and mineral density among the graft, leading us to question the theoretical advantages of the PRP with a bone grafts association for facial skeleton reconstruction [9].

However, the assumption that results from a PRP study from the endochondral bone of the rabbit tibia may be similar to the intramembranous bone of the facial skeleton is a large jump.

The PRP acts as a biological aggregating the particles, making it easier to handle the material, which resists to the movements during the flap closure and postoperative period. The literature review does not take a position about the PRP clinical usefulness, graft-combined or not, for treatment of bone defects to the aesthetic and functional rehabilitation. The PRP may have a strong clinical potential, but well-controlled clinical studies are needed to evaluate the highest concentration of different growth factors.

Further studies are needed to get out the PRP association with different bone substitutes and controlled delivery of growth factors could provide a beneficial effect for osteogenesis.

CONCLUSION

The graft particles resorption has been more powerful in the group PRPA than in the group A, during the 15 to 30-day periods. The PRP promoted greater vascularization in periods of the 15 to 30 days. The osteogenic tissue in the groups treated with the PRP was loose, while the untreated was fibrous. The PRP related or not to autologous bone graft, did not change on the biomechanical resistance and optical density of bone tissue formed that was higher in groups A and PRPA when compared to groups C and PRP. The results gained in this study allowed concluding that PRP, with or without autogenous bone graft, did not decide on the volume of the bone, and on the structural quality of bone tissue formed. This study showed no clinical benefits when PRP was added to grafts.

ACKNOWLEDGMENTS

We are grateful to the Bioscience Center for Special Health Care Needs, São José dos Campos Dental School, São Paulo State University. We also thank Dr. Ângelo Caporalli Filho and Dr. Aélcio Zangrandi, (Department of Materials and Technology Department, Guaratinguetá Engineering School, São Paulo State University) for biomechanical analysis contribution in this study.

REFERENCES

1. Macedo NL, Macedo LG, Monteiro ASF. Calcium sulfate and PTFE nonporous barrier for regeneration of experimental bone defects. Med Oral Patol Oral Cir Bucal. 2008 Jun;13(6):E375-9.
2. Jovanovic SA. Bone rehabilitation to achieve optimal aesthetics. Pract Proced Aesthet Dent. 2007 Oct;19(9):569-76.
3. Frechette JP, Martineau I, Gagnon G. Platelet-rich plasmas: growth factor content and roles in wound healing. J Dent Res. 2005 May;84(5):434-9.
4. Plachokova AS, Nikolidakis D, Mulder J, Jansen JA, Creugers NH. Effect of platelet-rich plasma on bone regeneration in dentistry: a systematic review. Clin Oral Implants Res. 2008 Jun;19(6):539-45.
5. Fontana S, Olmedo DG, Linares JA, Guglielmotti MB, Crosa ME. Effect of platelet-rich plasma on the peri-implant bone response: an experimental study. Implant Dent. 2004 Mar;13(1):73-8.
6. Boyapati L, Wang HL. The role of platelet-rich plasma in sinus augmentation: a critical review. Implant Dent. 2006 June;15(2):160-70.
7. Grageda E. Platelet-rich plasma and bone graft materials: a review and a standardized research protocol. Implant Dent. 2004 Dec;13(4):301-9.
8. Kassolis JD, Reynolds MA. Evaluation of the adjunctive benefits of platelet-rich plasma in subantral sinus augmentation. J Craniofac Surg. 2005 Mar;16(2):280-7.
9. Prakash S, Thakur A. Platelet Concentrates: Past, Present and Future. J Maxillofac Oral surg. 2011;10(1):45-9.
10. Hokugo A, Sawada Y, Hokugo R, Iwamura H, Kobuchi M, Kambara T, et al. Controlled release of platelet growth factors enhances bone regeneration at rabbit calvaria. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007 July;104(1):44-8.
11. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet-rich plasma: Growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1998 June;85(6):638-46.
12. Thorwarth M, Rupprecht S, Falk S, Felszeghy E, Wiltfang J, Schlegel KA. Expression of bone matrix proteins during de novo bone formation using a bovine collagen and platelet-rich plasma (prp) - an immunohistochemical analysis. Biomaterials. 2005 May;26(15):2575-84.
13. Lucarelli E, Fini M, Beccheroni A, Giavaresi G, Di Bella C, Aldini NN, et al. Stromal stem cells and platelet-rich plasma improve bone allograft integration. Clin Orthop Relat Res. 2005 June(435):62-8.
14. Landesberg R, Roy M, Glickman RS. Quantification of growth factor levels using a simplified method of platelet-rich plasma gel preparation. J Oral Maxillofac Surg. 2000 Mar;58(3):297-300; discussion 300-1.
15. Weibrich G, Hansen T, Kleis W, Buch R, Hitzler WE. Effect of platelet concentration in platelet-rich plasma on peri-implant bone regeneration. Bone. 2004 Apr;34(4):665-71.

16. Jensen TB, Rahbek O, Overgaard S, Soballe K. No effect of platelet-rich plasma with frozen or processed bone allograft around noncemented implants. Int Orthop. 2005 Apr;29(2):67-72.

17. Sarkar MR, Augat P, Shefelbine SJ, Schorlemmer S, Huber-Lang M, Claes L, et al. Bone formation in a long bone defect model using a platelet-rich plasma-loaded collagen scaffold. Biomaterials. 2006 Mar;27(9):1817-23.

18. Aghaloo TL, Moy PK, Freymiller EG. Evaluation of platelet-rich plasma in combination with anorganic bovine bone in the rabbit cranium: a pilot study. Int J Oral Maxillofac Implants. 2004 Jan./Feb;19(1):59-65.

19. Kim SG, Kim WK, Park JC, Kim HJ. A comparative study of osseointegration of Avana implants in a demineralized freeze-dried bone alone or with platelet-rich plasma. J Oral Maxillofac Surg. 2002 Sept;60(9):1018-25.

20. Choi BH, Zhu SJ, Kim BY, Huh JY, Lee SH, Jung JH. Effect of platelet-rich plasma (PRP) concentration on the viability and proliferation of alveolar bone cells: an in vitro study. Int J Oral Maxillofac Surg. 2005 June;34(4):420-4.

21. Ohya M, Yamada Y, Ozawa R, Ito K, Takahashi M, Ueda M. Sinus floor elevation applied tissue-engineered bone. Comparative study between mesenchymal stem cells/platelet-rich plasma (PRP) and autogenous bone with PRP complexes in rabbits. Clin Oral Implants Res. 2005 Oct;16(5):622-9.

22. Mazor Z, Peleg M, Garg AK, Luboshitz J. Platelet-rich plasma for bone graft enhancement in sinus floor augmentation with simultaneous implant placement: patient series study. Implant Dent. 2004 Mar;13(1):65-72.

23. Monov G, Fuerst G, Tepper G, Watzak G, Zechner W, Watzek G. The effect of platelet-rich plasma upon implant stability measured by resonance frequency analysis in the lower anterior mandibles. Clin Oral Implants Res. 2005 Aug;16(4):461-5.

24. Sommeling CE, Heyneman A, Hoeksema H, Verbelen J, Stillaert FB, Monstrey S. The use of platelet-rich plasma in plastic surgery: A systematic review. J Plast Reconstr Aesthet Surg. 2013;66(3):301-11.

25. Broggini N, Hofster W, Hunziker E, Bosshardt DD, Bornstein MM, Seto I, et al. The influence of prp on early bone formation in membrane protected defects. A Histological and Histomorphometric Study in the Rabbit Calvaria. Clin Implant Dent Relat Res. 2011;13(1):1-12.

26. Furst G, Gruber R, Tangl S, Zechner W, Haas R, Mailath G, et al. Sinus grafting with autogenous platelet-rich plasma and bovine hydroxyapatite. A histomorphometric study in minipigs. Clin Oral Implants Res. 2003 Aug;14(4):500-8.

27. Jakse N, Tangl S, Gilli R, Berghold A, Lorenzoni M, Eskici A, et al. Influence of PRP on autogenous sinus grafts. An experimental study on sheep. Clin Oral Implants Res. 2003 Oct;14(5):578-83.

28. Gerard D, Carlson ER, Gotcher JE, Jacobs M. Effects of platelet-rich plasma on the healing of autologous bone grafted mandibular defects in dogs. J Oral Maxillofac Surg. 2006 Mar;64(3):443-51.

29. Gerard D, Carlson ER, Gotcher JE, Jacobs M. Effects of platelet-rich plasma at the cellular level on healing of autologous bone-grafted mandibular defects in dogs. J Oral Maxillofac Surg. 2007 Apr;65(4):721-7.

30. Nather A, Thambyah A, Goh JC. Biomechanical strength of deep-frozen versus lyophilized large cortical allografts. Clin Biomech (Bristol, Avon). 2004 June;19(5):526-33.

31. Klongnoi B, Rupprecht S, Kessler P, Zimmermann R, Thorwarth M, Pongsiri S, et al. Lack of beneficial effects of platelet-rich plasma on sinus augmentation using a fluorohydroxyapatite or autogenous bone: an explorative study. J Clin Periodontol. 2006 July;33(7):500-9.

32. Plachokova AS, van den Dolder J, Stoelinga PJ, Jansen JA. The bone regenerative effect of platelet-rich plasma in combination with an osteoconductive material in rat cranial defects. Clin Oral Implants Res. 2006 June;17(3):305-11.

33. Fuerst G, Reinhard G, Tangl S, Mittlbock M, Sanroman F, Watzek G. Effect of platelet-released growth factors and collagen type I on osseous regeneration of mandibular defects. A pilot study in minipigs. J Clin Periodontol. 2004 Sept.;31(9):784-90.

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Date submitted: 2014 Apr 29

Accept submission: 2014 Oct 21