Original Article

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Evaluation of biomaterials with and without platelet-rich plasma: a histometric study using beagle dogs

Brunamelia de Oliveira[1], Wirley Gonçalves Assunção[1], Jacqueline Nelisis Zanoni[2], Edevaldo Tadeu Camarini[2], Roberta Okamoto[3]

[1]Universidade Estadual Paulista – UNESP, Araçatuba Dental School, Department of Dental Materials and Prosthodontics, Araçatuba, SP, Brazil
[2]Universidade Estadual de Maringá – UEM, Center of Biological Sciences, Department of Dentistry, Area of Morphological Sciences, Maringá, PR, Brazil.
[3]Universidade Estadual Paulista – UNESP, Araçatuba Dental School, Department of Basic Sciences, Araçatuba, SP, Brazil

Abstract

Aim: To compare the alveolar bone repair process using biomaterial in dogs with and without the incorporation of platelet-rich plasma. **Methods:** Six beagles were used. Bilateral extractions of the three mandibular premolars were performed. Bio-Gen® was applied in the first alveolus, the clot was maintained in the second alveolus and Genox® was applied to the third alveolus. PRP was added to all alveoli on the left side only. The dogs were submitted to euthanasia after 30, 60 and 90 days and submitted to histological analysis for the determination of mean area of new bone formation. Tukey's post test was used in the statistical analysis. **Results:** Significant increase in bone formation occurred in Bio-Gen® + PRP when compared with the other groups at 30 and 90 days. In the evaluation at 60 days, no statistically significant differences among the groups were found. **Conclusions:** The Bio-Gen® biomaterial led to the best bone repair and the combination of platelet-rich plasma accelerated the repair process.

Keywords: biocompatible materials; platelet-rich plasma; bone regeneration.

Introduction

The reconstruction of bone defects in the jaws has been widely studied[1-3]. The main objective is to achieve future oral rehabilitation with the use of dental implants. The correction of bone defects can be achieved with biomaterials, which are substances with combinations of a natural or synthetic origin indicated for the replacement of tissue. Biomaterials are currently used more often than homogenous, xenogenous and autogenous grafts[4-5].

A xenogenous graft is obtained from a donor of a different species. Bio-gen® (Bioteck- Arcugnano Vicenza, Italy) is a natural osteoconductive material of equine origin without collagen with a high osteogenic capacity due to the absence of calcination in the mixing phase, which preserves a large portion of the biological properties of this material. Complete resorption time ranges from four to 12 months[6]. Genox® (Baumer, São Paulo, SP, Brazil) is a product of bovine origin with an inorganic, freeze-dried matrix sterilized with gamma radiation. This product acts as an osteoconductor, conferring strength to the bone bed as it is resorbed[7].

Autogenous grafts are considered the gold standard due to the absence of

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Correspondence to:
Brunamélia de Oliveira
Rua Taioaba 125, Mooca, São Paulo
CEP: 03170-070 São Paulo, SP, Brasil
Phone: +55 44 9944-4984 / +55 11 99941-0010
E-mail: brunamoliveira@hotmail.com

immune reactions and disease transmission, as well as their innate osteogenic, osteoinductive and osteoconductive properties[8]. However, the disadvantages are the need for two simultaneous surgical sites and the risk of infection[9].

Platelet-rich plasma (PRP) is an autogenous substance used in the form of a platelet gel extracted through the centrifugation of a blend of bovine thrombin, 10% calcium chloride and venous blood[10-11]. PRP has a significant concentration of growth factors that enhance bone formation and mineralization, induce stem cells to differentiate into osteoblasts, diminish bone resorption, promote angiogenesis and produce collagen through the activation of fibroblasts[12-13]. A number of studies have demonstrated that the combination of PRP and biomaterials achieves the best results in the bone repair process in comparison to groups in which PRP was not employed[14-15].

The purpose of the present study was to perform bilateral tooth extractions in dogs and compare the alveolar bone repair process using Bio-gen® (Bioteck) and Genox® (Baumer) with and without the incorporation of platelet-rich plasma.

Materials and methods

Six beagles with a mean age of two years and weighing approximately 10 kg were maintained in appropriate confinement for the species (two dogs per kennel) in a clean, aerated environment with free access to food and water.

The procedures employed in this study were approved by the Ethics Committee on Animal Experimentation (CEUA/EMU) - State University of Maringá, process number 017/2010.

Preparation of PRP

Forty milliliters of blood were taken from the jugular vein of each animal. Sodium citrate 10% was used as anticoagulant. The blood was homogenized and centrifuged at 1200 rpm for 10 min (SIN centrifuge: SIN implant system, São Paulo, SP, Brazil). The first centrifugation resulted in the complete separation of the blood into two layers: a lower layer of red blood cells and an upper layer of plasma. The plasma layer had two parts: the upper portion (approximately 75% of the total plasma) had a greater concentration of platelet-poor plasma (PPP) and the lower portion (25%) had a greater concentration of PRP. For the second centrifugation, the total plasma (PPP and PRP) was pipetted and transferred to a new recipient, which was centrifuged at 1200 rpm for 10 min for better separation of both parts. The lower portion (containing PRP rich in growth factors) was then pipetted[1].

The gelification process involved the use of a 10-mL syringe for each mixture, with 6 mL of PRP, thrombin, calcium gluconate and air to assist in the mixture. The syringe was manually shaken for 6 to 10 s until the gel was formed.

Surgical procedure

The animals were pre-medicated with acepromazine 0.2% at a dose of 0.03 mg/kg and fentanyl citrate at a dose of 3 µg/kg intravenously with the administration of Ringer's lactate solution at a volume of 10 mL/kg/h to maintain the venous access. Anesthesia was performed with propofol at a dose of 3 mg/kg and 2 mg/kg of ketamine intravenously and a local block with 0.5% bupivacaine. Systolic blood pressure was monitored using vascular Doppler. Intubation was performed using a number 7.5 tube and oxygen flow was maintained at 1 mL/kg/h.

Bilateral extraction of the mandibular three premolars was performed on all dogs without compromising the alveolar crest. On the right side, Bio-Gen®(Bioteck) was applied in the first alveolus, the clot was maintained in the second alveolus and Genox®(Baumer) was applied to the third alveolus. On the left side, Bio-Gen®(Bioteck) + PRP was applied in the first alveolus, the clot was maintained and PRP was applied to the second alveolus and Genox®(Baumer) + PRP was applied to the third alveolus (Figure 1A). The alveoli were completely filled with the biomaterials. When PRP was added to the grafts, each was mixed individually. The suture was performed with Vicryl 4.0 (Ethicon, Johnson & Johnson, Somerville, NJ, USA) (Figure 1B).

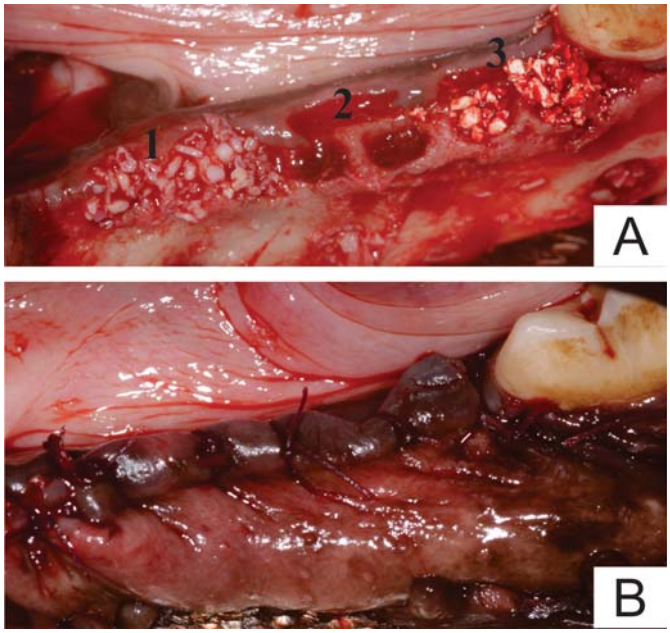

Fig. 1. Left side of beagle mandible: A) 1- First alveolus – Bio-gen® + PRP; 2- Second alveolus – clot + PRP; 3- Third alveolus – Genox® + PRP; B) Suture with single stitch recovering alveolar ridge.

Post-extraction care

After the surgical procedures, the animals received an intramuscular injection of 1 mL of enrofloxacin 5% (5 mg/kg, Baytril®) and an intravenous dose of 5 mL of dipyrone 50%. Over the next three days, the animals received 1 mL/10 kg oral doses of dipyrone 50%.

At 30, 60 and 90 days postoperatively, two animals were euthanized with an intravenous dose of thiopental (25 mg/kg), fentanyl (3 µg/kg and potassium chloride (20 mL). The mandibles were dissected and each alveolus studied was submitted to histological analysis with hematoxylin-eosin staining.

Histomorphometric study

Four cuts/alveolus/animal/time were selected for the analysis of bone formation. Five images were obtained for each cut through the center of each alveolus following the long axis and using the adjacent alveolar bone as reference (Figure 2). The images (total area: 5.56 mm^2) were captured using a camera (QColor3; Olympus, Tokyo, Japan) coupled to an optical microscope (BX41, Olympus) with a 4x objective. The histomorphometric measures were made with the Image Pro Plus® software, version 4.5 (Media Cybernetics, Rockville, MD, USA). A polygonal measuring tool was used to delimit the area of mature bone on each image. The mean area of newly formed bone was measured on each cut.

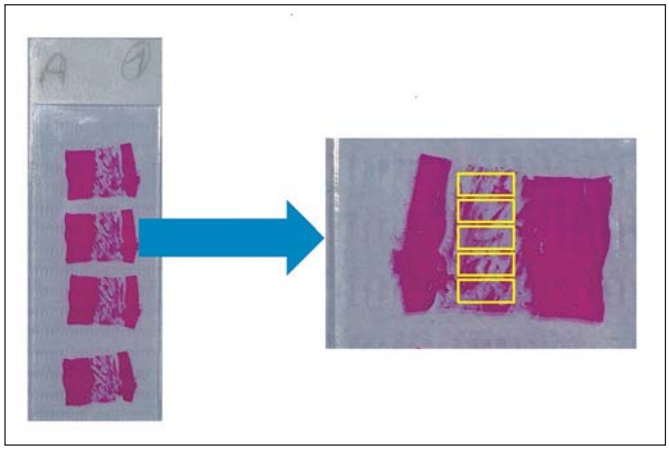

Fig. 2. Images of center of each alveolus following long axis with adjacent alveolar bone as reference.

Statistical analysis

The data were analyzed using the GraphPad Prism R 3.1 (GraphPad Software, Inc. La Jolla, CA, USA). Nonparametric one-way analysis of variance (ANOVA) was employed. Tukey's post hoc test was used for the comparison of means, with the level of significance set to 5% ($p<0.05$).

Results

Table 1 displays the mean area of newly formed bone in the alveoli of the different groups [clot, Bio-gen® (Bioteck), Genox®(Baumer), clot + PRP, Bio-gen® (Bioteck) + PRP and Genox® (Baumer) + PRP] at the different evaluation times (30, 60 and 90 days). At 30 days, greater bone formation was found in all groups in comparison to the control (clot).

Table 1. Mean area (mm^2) of newly formed bone in different groups at different evaluation times

	Bio-gen®	Clot	Genox®	Bio-gen® + PRP	Clot + PRP	Genox® + PRP
30 days	0.9 ^{b}	0.58 ^{b}	1.24	1.51 ^{a}	0.91 ^{b}	1.22
60 days	3.17	2.62	2.91	2.92	2.08	2.92
90 days	3.64	2.16 ^{b}	3.17 ^{b}	4.59 ^{a}	1.82 ^{b}	2.36 ^{b}

Means with different letters denote statistically significant differences among groups in each period. Tukey post hoc test at 5% is considered significant level ($p<0.05$).

The best result was achieved with Bio-gen®(Bioteck) + PRP, with statistically significant differences in comparison to the clot, clot + PRP and Bio-gen®(Baumer) groups ($p<0.05$)

At 60 days, an increase in bone formation was found for all groups compared with the 30-day evaluation. However, no significant differences were found among the different groups (Table 1).

At 90 days, the lowest rates of bone formation were found in the clot groups and the best result was achieved with Bio-gen® (Bioteck) + PRP, which differed significantly from all other groups ($p<0.05$), except the Bio-gen® (Bioteck) group (Table 1).

Figure 3 shows the bone formation rate in the groups with PRP at the different evaluation times. Better results were achieved with Bio-gen® (Bioteck) + PRP in comparison to clot + PRP and Genox® (Baumer) + PRP. However, the difference was only statistically significant at the 90-day evaluation ($p<0.05$). Figure 4 displays the results of the groups without PRP at the different evaluation times.

Figure 5 shows the histological cuts (4× magnification). Greater bone formation was evident in the Bio-gen® (Bioteck) + PRP in comparison to all other groups at the 90-day evaluation.

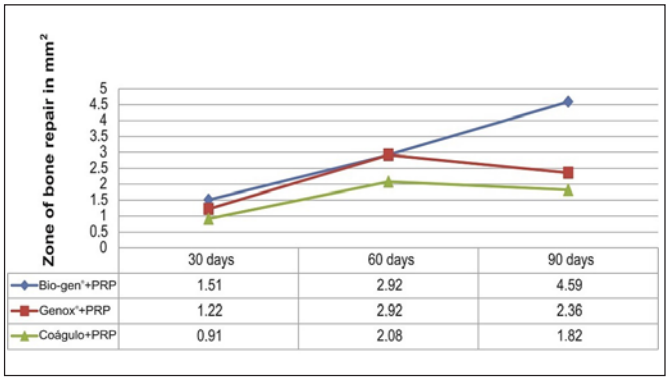

Fig. 3. Mean area of bone formation (mm^2) at 30, 60 and 90 days in the alveoli of clot + PRP, Genox® + PRP and Bio-gen® + PRP groups.

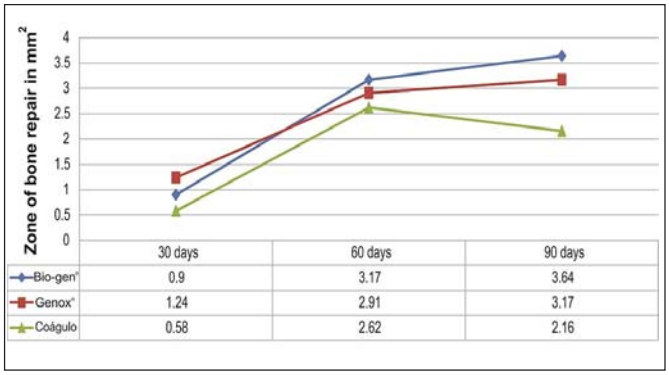

Fig. 4. Mean area of bone formation (mm^2) at 30, 60 and 90 days in the alveoli of clot, Genox® and Bio-gen® groups.

Discussion

A number of studies in the literature have demonstrated the biocompatibility of deproteinized animal matrices used

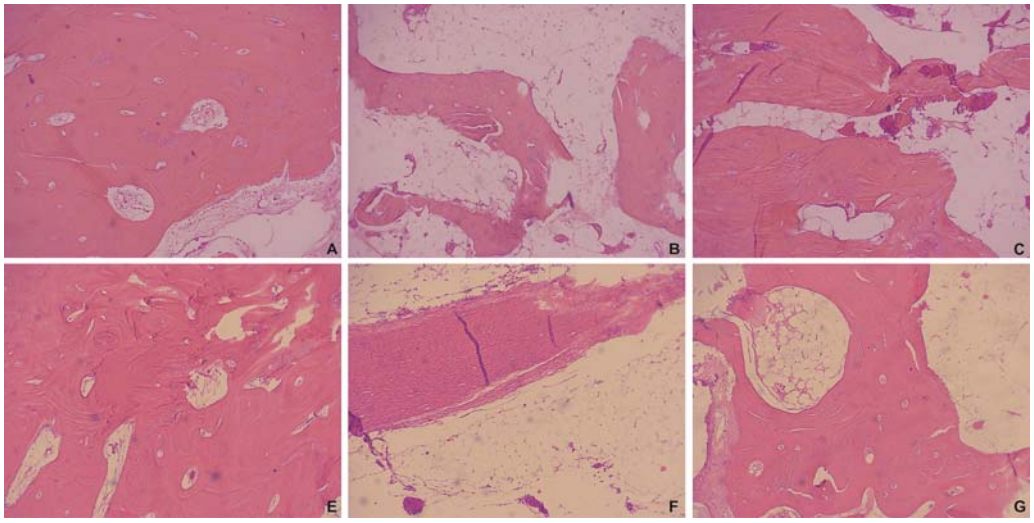

Fig. 5. Histological cuts (4´ magnification) of alveoli of mandibular premolars in beagles 90 days after extraction (hematoxylin-eosin stain): A) Bio-gen®; B) clot; C) Genox®; E) Bio-gen® + PRP; F) clot + PRP; G) Genox® + PRP.

as bone graft material[16-17]. In the present nvestigation, Genox® (Baumer) exhibited a tendency toward greater bone formation in comparison to Bio-gen® (Bioteck) at the 30-day evaluation. This finding may be explained by the fact that the surface of this material exerts an influence on cell colonization due to its preserved micro-architecture, which offers spaces for cells to fill, thereby enabling angiogenesis, cell migration, adhesion and the formation of new bone tissue. Martins et al.[18] (2004) report similar results regarding a bone graft of bovine origin. In the same evaluation period, the addition of PRP to Genox® (Baumer) did not enhance the bone formation process. This finding agrees with data reported by Rocha et al.[19] (2010), who report that the combination of PRP and Genox® (Baumer) did not lead to a significant increase in bone formation. A possible explanation for this would be the fact that PRP has a longer action time (up to seven days after preparation) and biomaterial of bovine origin is made by deproteinization at high temperatures, causing permanent changes, which may decrease the action of the growth factors in PRP[20].

The best results at the 30-day evaluation were achieved when PRP was added to Bio-gen® (Bioteck), with a significant increase in bone formation in comparison to the use of Bio-gen® (Bioteck) alone. This likely occurred due to the mixing process, which is performed at 37 °C, with decontamination occurring through enzymatic means and sterilization by gamma-radiation[21]. This process is less aggressive to the structure of the proteins in the biomaterial. Thus, the more active growth factors in PRP probably led to this finding.

The worst results were found in the clot groups with and without PRP at all evaluation times. This may be related to the progressive atrophy of the alveolar process that occurs following a tooth extraction[22-23]. Similar findings are described by Nevins et al.[24] (2006), who evaluated the width of the alveolus 30 and 90 days after extraction using computerized tomography (transverse cut) and demonstrated progressive atrophy of the bone. The authors concluded that filling the post-extraction alveolus with a biomaterial is a way to avoid this atrophy.

No statistically significant differences among the different groups were found at the 60-day evaluation. This may be explained by the high degree of repair activity in the initial days following an extraction, with a subsequent reduction in the repair rate over time[25-26].

At the 90-day evaluation, the best results were achieved with Bio-gen® (Bioteck) + PRP, with a significantly greater rate of new bone formation in comparison to all other groups, except Bio-gen® (Bioteck) without PRP. This finding may be explained by the hydrophilic nature of Bio-gen® (Bioteck) and its structure, which allows cell migration and angiogenesis, thereby facilitating bone regeneration. Moreover, the production of this material at low temperature allows eliminating organic matter without causing changes in the structure of trabecular bone[27]. In contrast, Genox® (Baumer) is derived from de-mineralized, hydrophobic bovine bone composed only by inorganic matrix[28-29].

Smieszek-Wilczewska et al.[30] (2010) compared Bio-gen® (Bioteck), Bio-oss® and a control group (clot alone). The authors found that Bio-gen® (Bioteck) led to a significantly greater increase in radiological density compared with the other groups, confirming the efficacy of biomaterial of equine origin.

Considering the importance of preserving the post-extraction alveolus for future interventions such as implants, the present findings demonstrate that Bio-gen® (Bioteck) is an excellent biomaterial for the repair and conservation of alveolar bone structures. Moreover, the results were even more satisfactory when this product was used together with PRP, as demonstrated by the accelerated bone formation process. Further studies should compare the structure of biomaterials of equine and bovine origin to confirm the advantage of one material over the other.

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