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Guided bone regeneration with polypropylene barrier in rabbit's calvaria: A preliminary experimental study

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Abstract

Objectives: This study aimed to evaluate the bone formation process in experimental defects created on rabbit calvarial, in which one of the bone defects was covered by the impermeable membrane before suturing the skin flap, while the other was closed only by the cutaneous flap. The experimental holes were filled only by the blood clot.

Material and methods: Sixteen New Zealand female rabbits weighing between 3.5 and 4 kg were used. Two experimental bone defects were made in the rabbit calvarial. The holes were filled only with the blood clot and one of them was covered with an impermeable polypropylene membrane. A histological analysis was made at 21 and 42 days following the surgery. Histological evaluation consisted of the following: 1. inflammatory process; 2. Bone repair; 3. Bone remodeling; 4. Presence of osteoid matrix and mineralization, and 5. Formation of hematopoietic tissue. Each characteristic was analyzed semi quantitatively.

Results: There was a statistical difference between the test and the control group at 21 days of healing in the following items: presence of cementation line ($p = 0.012$), presence of osteoid tissue ($p = 0.012$), and trabecular bone tissue development and

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mineralization ($p = 0.012$). A greater amount of lamellar bone tissue (mature) was also observed in the test group compared to the control group.

Conclusion: The semiquantitative analysis showed that at 21 days there was a superiority of the repair process in the test group; at 42 days there was no significant difference in bone formation between the two groups; and that the polypropylene membrane is feasible to be used in GBR.

Clinical significance: The impermeable polypropylene barrier is feasible for use in the guided bone regeneration technique. It can be used only on the blood clot, without the need for grafting, and can be easily removed a few days after surgery. These results are unprecedented.

Keywords: Dentistry, Materials science

1. Introduction

The process of tissue healing is of great importance in Medicine and Dentistry, especially when there are clinical conditions that interfere in this process. The process of bone formation that occurs after tooth extraction in the residual socket is an example. It is important to preserve or reduce post-extraction alveolar bone loss, which is unavoidable [1] and may hamper oral rehabilitation by causing severe complications when considering dental implants in the future [2]. This can be one of the great challenges of modern dental implantology.

It has been known for a long time that the reduction of socket dimensions after tooth extraction is higher in the first year and that this continues with time [3]. Several factors are associated with alveolar absorption, local and systemic, and loss of alveolar bone may be greater when there is damage to the socket walls before or during the extraction procedure.

Several techniques have been described to preserve the dimensions of the postoperative socket and one of the most efficient is called Guided Bone Regeneration (GBR). Its basic principle is the use of a membrane that acts as a physical barrier to prevent the proliferation of epithelial cells within the physical space of the socket filled with the blood clot, which could delay and or prejudice the bone regeneration [4, 5, 6, 7]. Different types of barrier membranes have been described in the last 30 years: resorbable, non-resorbable, with greater or lesser permeability, with primary closure of gingival flaps by suture [8, 9, 10, 11, 12, 13] or exposed to the oral environment to be removed without a second surgical procedure [14, 15], and with or without graft material within the socket. Many of these barriers are currently marketed and widely used for the preservation of alveolar bone.

Although membrane porosity was considered a prerequisite for GBR, a study using impermeable barrier showed that permeability is not required for bone formation

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using this technique [16]. On the other hand, reports of clinical cases suggest that polypropylene impermeable membranes also contribute to the preservation of the alveolar ridge after tooth extraction, especially in cases where the buccal bone wall of the socket was damaged previously or during tooth extraction [17, 18, 19, 20, 21]. Thus, the present study aimed to evaluate the bone formation process in experimental defects created in calvaria of rabbits, in which one of the defects was covered by the impermeable membrane before suturing the skin flap, while the other was closed only by the cutaneous flap. The experimental holes were filled only by the blood clot.

2. Material and methods

Sixteen New Zealand female rabbits weighing between 3.5 and 4 kg; aged between 11 and 15 months underwent calvaria surgery in the facilities of the laboratory of multidisciplinary surgical technique of the university. The rabbits were kept under controlled ventilation and temperature (22 ± 2 °C) and fed with feed (Nutríara, São Paulo-Brazil) and water *ad libitum*.

2.1. Surgical procedure

Veterinary nursery staff according to the following protocol performed anesthesia: Ketamine IM (30 mg/kg), Xilasin IM (5 mg/kg), and Meperidine IM (5 mg/kg). Rabbits received oxygen by mask throughout the procedure, and the respiratory and cardiac frequencies were monitored by an oximeter. The tricotomy of the surgical area was performed with an electric razor and the antisepsis with a 2% chlorhexidine solution (Rioquímica, São José do Rio Preto, Brazil). The cutaneous incision was performed with a no. 15 surgical scalpel blade on the medial portion of the calvaria through a full thickness flap. The periosteum was detached and two critical bone defects/holes were prepared, one on each side of the calvaria (Fig. 1). The holes were

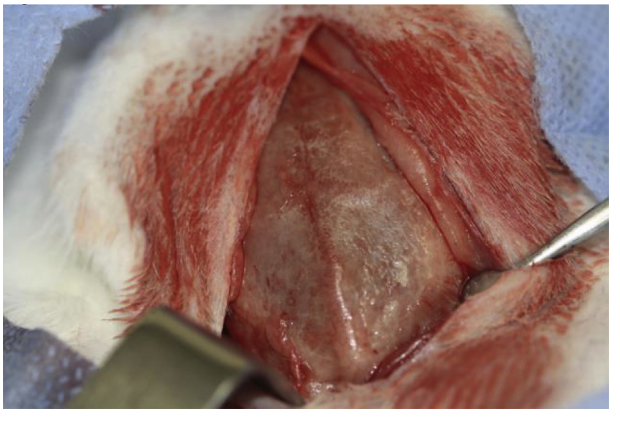

Fig. 1. The periosteum was detached leaving the calvaria exposed.

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made through a 6.25 mm diameter trephine and an 8.8 mm spherical diamond drill, which were mounted on a contra-angle handpiece for dental implant under constant irrigation with sterile physiological solution (Fig. 2). After drilling 1 mm with the trephine to demarcate the defect width, the spherical drill was used to deepen it, taking care neither to damage the internal cortical of the calvaria nor expose the dura mater. After filling the bone defects with the rabbit own blood, one of them was covered with a polypropylene membrane (INP System, São Paulo/Brazil), which was fixed to the bone by thumbtacks (Fig. 3), followed by suture of the skin flap. Only the skin flap suture covered the other defect. After the surgeries, the animals received antibiotic (Enrofloxacin – 5–10 mg/kg/IM) and non-steroidal anti-inflammatory drugs (Meloxican- 0.2 mg/kg/IM) and were monitored until their total recovery.

After 21 or 42 days following the surgery, the rabbits were anesthetized through the same protocol described above. The calvaria area was exposed and the polypropylene membrane was removed (Fig. 4). The bone tissue formed in the experimental hole was collected by osteotomy using a 13 mm trephine drill (Fig. 5). The animals were put down using Thiopental IV (20 mg/kg) and Potassium Chloride (19.1%, 1

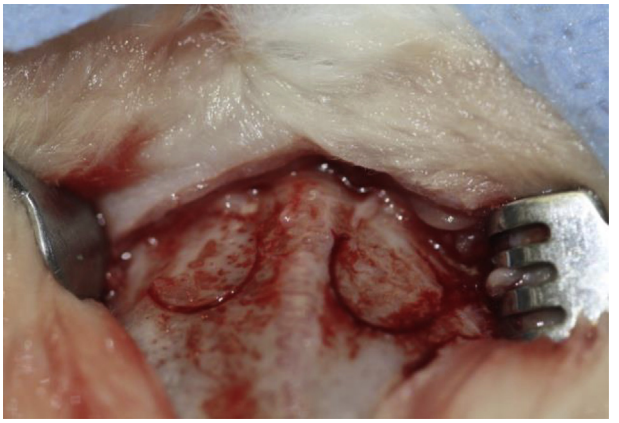

Fig. 2. Demarcation of both holes in the calvaria.

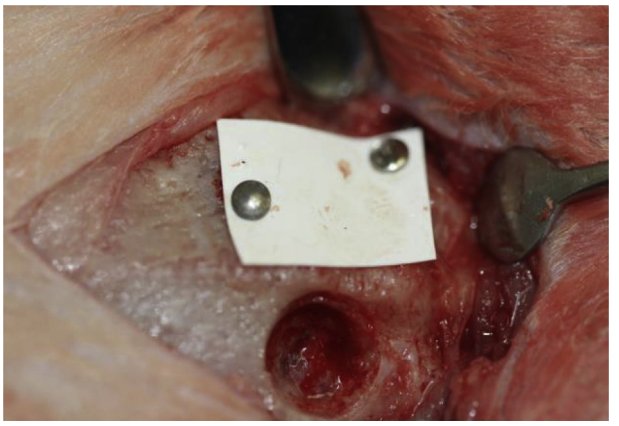

Fig. 3. The polypropylene membrane was attached to the bone by thumbtacks.

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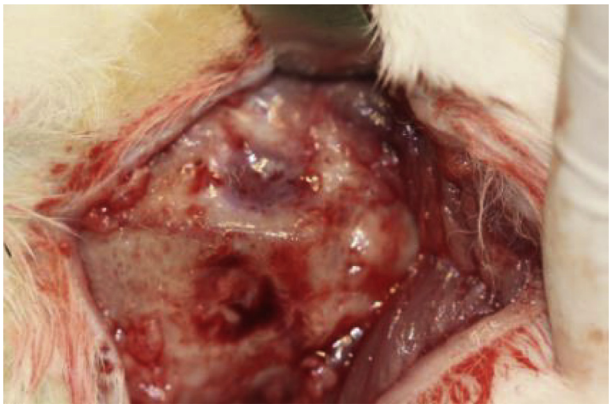

Fig. 4. The calvaria was exposed and the polypropylene membrane was removed (upper area).

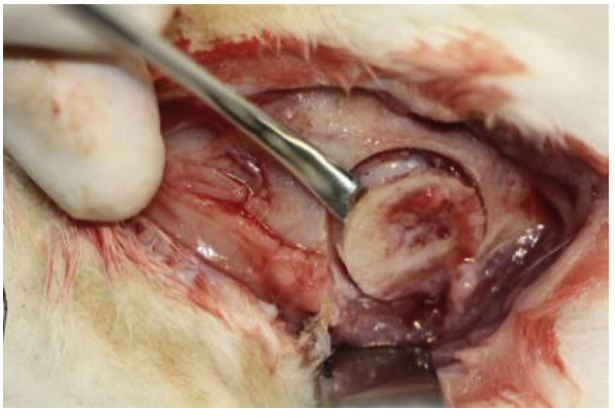

Fig. 5. The bone tissue formed in the experimental hole was collected by osteotomy.

ampoule/animal). The specimens were fixed in 10% paraformoldehyde, decalcified for four days in a 20% aqueous solution of sodium citrate and 12.5% aqueous solution of formic acid before being embedded in paraffin. Part of the samples were staining with hematoxylin/eosin (HE) for conventional analysis of the bone repair process and part with Masson's trichrome (MT) for better tissue differentiation and to reveal the presence of collagen.

The samples were divided into two groups: Control group, without membrane; and Test group, which received the polypropylene membrane. For the histological analysis the groups were blinded and the examiner (an independent bone pathologist) did not know to which group the sample belonged. They were evaluated using the optical microscope (Opticam O400S) and the histological evaluation consisted of the analysis of the following steps of the bone repair process [22]:

1. Inflammatory process: inflammatory infiltrate, hemorrhage, edema and granulation tissue formation; 2. Bone repair: angiogenesis, presence of active fibroblasts, periosteal and endosteal reaction; 3. Bone remodeling: compact bone tissue (activation phases, osteoblastic reabsorption, osteoblastic reversal and formation of

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lamellar bone or osteons), and trabecular (spongy) bone tissue (with the same phases of the compact bone); 4. Presence of osteoid matrix and mineralization; and 5. Formation of hematopoietic tissue. Each characteristic mentioned above was analyzed semi quantitatively according to the degrees of intensity namely: absent (−), discrete (+), moderate (++) and accentuated (+++).

2.2. Statistical analysis

For the statistical analysis of results, the Fisher's exact test was used to compare the control and test groups in relation to the degrees of intensity (high and low) for each step in the bone repair process. In all tests the level of significance was set at 0.05 or 5%. The Research Ethics Committee of the University of Santo Amaro/São Paulo/Brazil (n o 118/2009) approved the project in accordance with the principles guiding biomedical research involving animals.

3. Results

There were no complications in the postoperative period. Macroscopically there was no dehiscence of the skin suture, signs of local or systemic infection, or even extrusion of the implanted membrane. At the time of sample collection, there was no interposition of soft tissue between barrier and bone tissue formed. Experimental holes in the test group showed a more rigid consistency, with a more organized, hardened and less fibrous tissue, when compared to the holes of the control group, in both periods of 21 and 42 days of repair, respectively. Compared with neighboring bone tissue, the test defect tissue was less compact, typical of osteoid tissue.

3.1. Histological evaluation

The semiquantitative evaluation of the histological data of each animal are presented in Tables 1, 2, 3, and 4. In these, it can be observed that the bone formation process was slower in the control group, in comparison with the test group. There was a statistical difference between the test and the control group at 21 days of healing in the following items: presence of cementation line ($p = 0.012$), presence of osteoid tissue ($p = 0.012$), and trabecular bone tissue development and mineralization ($p = 0.012$). A greater amount of lamellar bone tissue (mature) was present in the test group compared to the control group. Histological evaluation is presented in Figs. 6, 7, 8, 9, 10, 11, 12, and 13.

4. Discussion

The literature on Guided Bone Regeneration (ROG) is vast, but there are still many points to be researched and discussed, such as what would be the best barrier to use

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Table 1. Control group (21 days). Quantitative analysis of the rabbits calvarial bone defect without polypropylene membrane.

Animal number/Histology	C11	C56	C57	C59	C60	C61	C63	C64
Inflammatory reaction	+	+	+	+	++	+	+	+
Cementant line	+	+	+	+	+	+	+	+
Fibrous tissue	++	++	++	++	+++	++	+++	++
Osteoid tissue	+	+	+	+	+	+	+	+
Hematopoietic tissue	+	+	+	+	−	+	−	−
Trabecular bone tissue	−	+	+	−	−	+	−	−
Cortical bone tissue	+	+	+	+	−	+	+	+

Intensity: absent (−); discret (+), moderate (++) and accentuated (+++).

Table 2. Test group (21 days). Quantitative analysis of the rabbits calvarial bone defect covered with polypropylene membrane.

Animal number/Histology	T11	T56	T57	T59	T60	T61	T63	T64
Inflammatory reaction	+	+	+	+	−	+	+	−
Cementant line	++	+	+	++	+	++	++	++
Fibrous tissue	++	++	++	++	+	++	++	+
Osteoid tissue	++	+	++	++	+	++	+	++
Hematopoietic tissue	+	+	+	+	+	+	+	+
Trabecular bone tissue	+	+	+	+	++	+	+	+
Cortical bone tissue	+	+	+	+	+	+	+	+

Table 3. Control group (42 days). Quantitative analysis of the rabbits calvarial bone defect without polypropylene membrane.

Animal number/Histology	C21	C22	C25	C26	C27	C29	C54	C55
Inflammatory reaction	−	−	−	−	−	−	−	−
Cementant line	++	+	+	++	+	++	+	++
Fibrous tissue	++	++	++	++	+	++	++	++
Osteoid tissue	++	+	+	+	++	++	++	+
Hematopoietic tissue	++	+	++	++	++	++	++	++
Trabecular bone tissue	++	++	++	++	+++	++	+++	++
Cortical bone tissue	++	++	++	++	+++	++	+++	++

Intensity: absent (−); discret (+), moderate (++) and accentuated (+++).

[23]. Studies with PTFE barriers indicate that they promote bone tissue growth with dense quality, while resorbable barriers can degrade and cause local inflammation, reducing bone formation [24]. The need for a second surgical time for removal of nonabsorbable barriers contained in primary closure represents also a drawback

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Table 4. Test group (42 days). Quantitative analysis of the rabbits calvarial bone defect covered with polypropylene membrane.

Animal number/Histology	T21	T22	T25	T26	T27	T29	T54	T55
Inflammatory reaction	–	–	–	–	–	–	–	–
Cementant line	++	+	+	++	++	++	++	++
Fibrous tissue	+	++	+	+	++	+	++	++
Osteoid tissue	++	+	++	++	+	++	++	++
Hematopoietic tissue	++	+	++	++	+	++	++	++
Trabecular bone tissue	++	+	++	++	++	++	++	++
Cortical bone tissue	+++	++	++	+++	++	+++	+++	+++

Intensity: absent (−); discret (+), moderate (++) and accentuated (+++).

for their use [13]. On the other hand, resorbable membranes can compromise the isolation of the area to be repaired [24].

The present study opted for a non-resorbable polypropylene barrier, considering its availability, biocompatibility and biofunctionality. The experimental defects were filled only with the blood clot. In contrast to other studies that used the removal of both bone cortices to make the rabbit calvarial defect [24, 25, 26, 27], we chose to maintain the internal cortical because this technique is less traumatic and prevents the loss of animals due to neurological complications [23].

In relation to the evaluation periods, there is great variation in the scientific literature, ranging from seven days to several months [23, 28, 29, 30, 31, 32, 33, 34, 35]. The cycle of bone remodeling in rabbits is six weeks, different from dogs (12 weeks) and humans (17 weeks). Thus, after the six-week period, changes in rabbits would already allow some stabilization, not interfering with the outcome of the analysis in question.

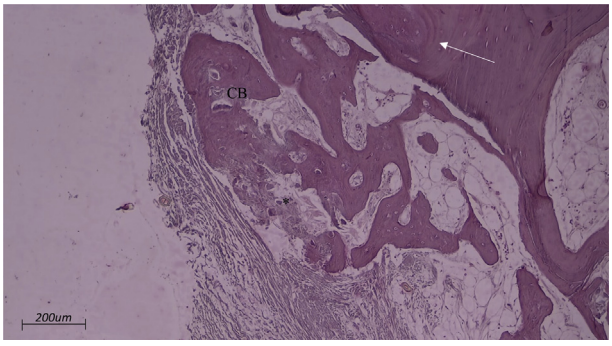

Fig. 6. Histology of new bone formed within the defect at the 21 days of the control group. Discrete presence of inflammatory cells (*). Fibrous areas, sometimes between the trabecular bone. Cortical bone tissue poorly developed but with some mineralization. Very visible cement lines in areas of osteoid matrix (arrow). HE, 100×.

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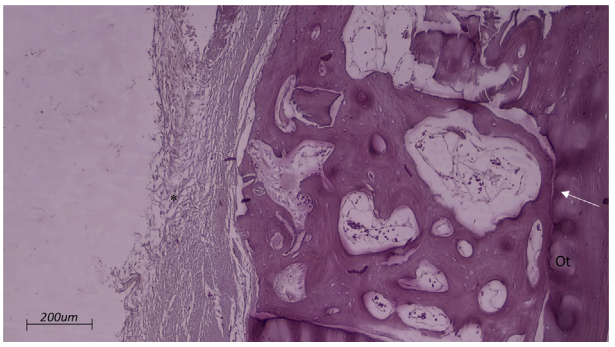

Fig. 7. Histology of new bone formed within the defect at the 21 days of the test group. Discrete presence of inflammatory cells (*). Areas with fibrous tissue. Trabecular and cortical bone tissues with moderate amount of osteoid tissue, and irregular osteons (Ot). Very visible cementing lines. HE, 100×.

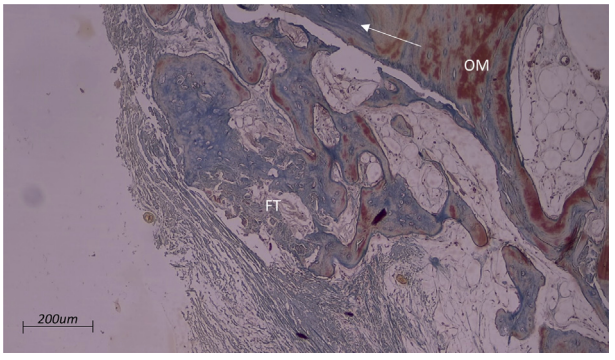

Fig. 8. Histology of new bone formed within the defect at the 21 days of the control group. Poorly mineralized cortical bone (CB). Frequent presence of areas of osteoid matrix (low calcified - brown areas) in developing bone tissue (OM). Visible cementitious lines (arrow). Moderate presence of fibrous tissue (FT). MT, 100×.

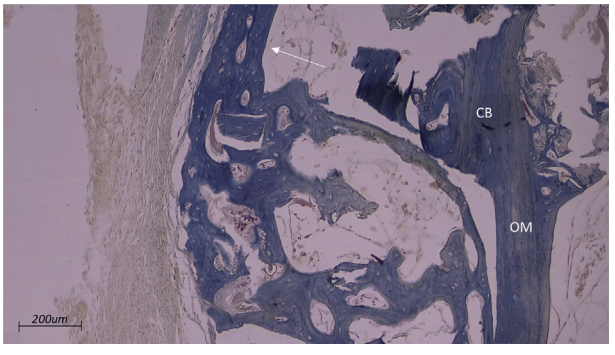

Fig. 9. Histology of new bone formed within the defect at the 21 days of the test group. Presence of cortical and trabecular bone tissues in formation with areas of osteoid matrix in discrete degree (brown areas) (OM). Moderate formation of fibrous tissue with discrete inflammatory infiltrate. Osteoblasts layer (arrow). MT, 100×.

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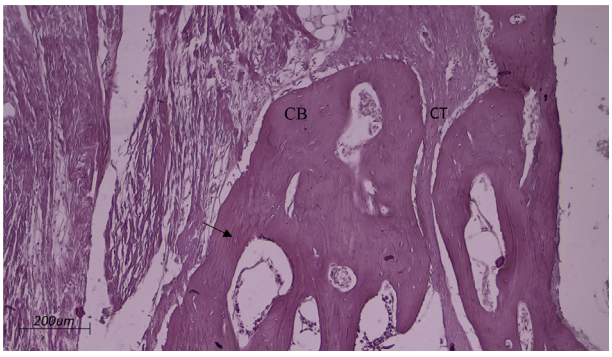

Fig. 10. Histology of new bone formed within the defect at the 42 days of the control group. Cortical bone development (CB) with initial osteons. Collagenous tissue (CT), intratrabecular. Cement line (arrow). HE, 100×.

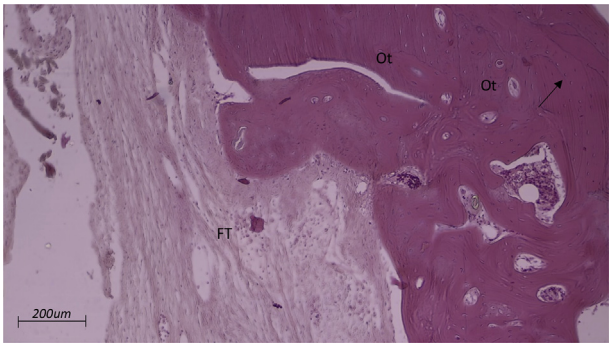

Fig. 11. Histology of new bone formed within the defect at the 42 days of the test group. Hypocellular peripheral fibrous tissue (FT). Very well developed cortical tissue with prominent osteons (Ot). Cement lines (arrow). Osteoid matrix with initial osteons (Ot). HE, 100×.

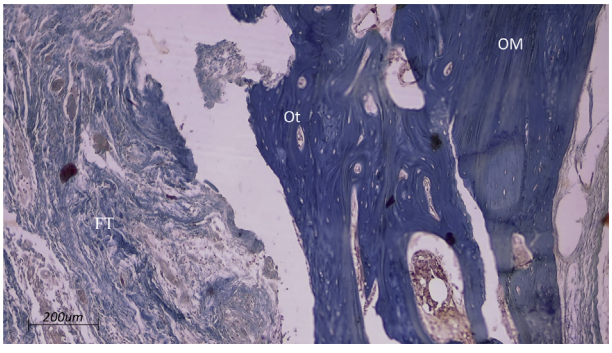

Fig. 12. Histology of new bone formed within the defect at the 42 days of the control group. Osteons (Ot). Hypocellular peripheral fibrous tissue (FT). Well mineralized cortical bone tissue with some osteoid matrix (OM). MT, 100×.

Although the gingiva and oral environment present distinct histology of the scalp, the rabbit calvarial models are very useful for the study of the technique of guided bone regeneration, since the bone of the craniofacial area presents common characteristics of healing. This model is well known and used with diverse types of materials and techniques [23, 24, 25, 26, 27, 28, 29, 30].

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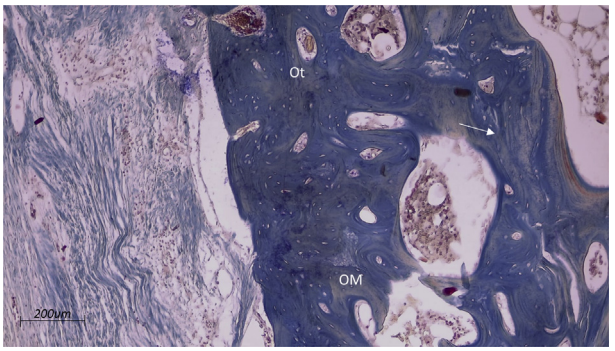

Fig. 13. Histology of new bone formed within the defect at the 42 days of the test group. Osteons with osteocytes (Ot). Peripheral fibrous tissue with hemorrhagic foci. Mineral cortical bone tissue with osteoid matrix (OM). Cement lines (arrow) MT, 100×.

Histologically, at 21 days of repair, in both groups the samples still presented some inflammatory evidence, which can be considered physiological. However, there was no significant difference between the two groups in relation to the inflammatory reaction, evaluated by either the cellular infiltrate, edema, hemorrhage or angiogenesis, indicating no foreign body response to the membrane. The periosteal reaction was marked in almost all the samples, thus demonstrating, independently of the group, a great activity of the periosteum during the osteogenesis.

A significantly higher presence of cementation line was observed in the test group compared to the control at 21 days of repair ($p = 0.012$). These lines separate the osteons from the interstitial lamellae and indicate a faster osteogenesis in this group [22]. A greater amount of fibrous tissue was present in the control group, both at 21 days ($p = 0.012$) and 42 days of repair, although the latter was not statistically significant. This difference can be explained by the non-isolation of the bone defects in this group, giving them a greater tissue competition, contrary to the test group. The osteoid tissue is a newly synthesized bone extracellular matrix and was gradually deposited as bands or lamellae in a significantly larger amount in the test group at 21 days of repair as compared to the control group ($p = 0.012$). This fact shows that osteoblasts probably became osteocytes more rapidly in the test group due to premature calcification of this matrix. These indications may confirm that in the test group there is acceleration of the mineral deposition in the osteoid matrix, mainly in osteogenesis of the trabecular bone.

In the process of intramembranous ossification, as occurs in flat bones of the skull, numerous ossification centers develop and eventually merge into a network of anastomosed trabeculae that resemble a sponge: it is spongy or trabecular bone tissue. This tissue was significantly larger in the test group at 21 days of repair, confirming the acceleration of osteogenesis in this group ($p = 0.012$). There was structural difference at 21 days of repair, both in the test and in the control group, regarding

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osteogenesis of the cortical bone. In the control, it was thinner and sometimes absent, while in the test group samples of a more solid formation occurred, characterizing a well formed and more mineralized laminar bone with osteocyte entrapment.

There were few changes in osteons. The test group, at both 21 and 42 days of repair, presented slightly more mature elements (with concentric lamellae in greater quantity), which could be explained by a greater acceleration of reversal phase or osteoblastic formation. These qualitative histological differences evidenced mainly at 21 days of repair make the study motivating, since they illustrate the fact that the GBR technique can accelerate the osteogenesis. Although this study shows favorable results for the use of the polypropylene membrane, further studies are still needed to confirm the initial perspective of the advantages of this material over other materials.

This is a semi-quantitative study, so it is necessary to analyze the results according to this important limitation. This experiment should be repeated and amplified to increase the groups and more complete testing using this type of membrane. Hystomorphometric studies should be done to assess the amount of actual bone growth between the groups.

5. Conclusion

According to the methodology of this study we can conclude the following: the inflammatory response was similar in both groups, while the semiquantitative analysis showed that at 21 days there was an superiority of the repair process in the test group, as evidenced by the presence of cementing line, osteoid tissue, less invasion of fibrous connective tissue and development and mineralization of trabecular bone structures; at 42 days there was no significant difference in bone formation between the two groups; and that the polypropylene membrane is feasible to be used in GBR.

Declarations

Author contribution statement

Leandro De Lucca: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Márcio da Costa Marques: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Ilan Weinfeld: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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