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Critical size defect regeneration by rhPTH-collagen membrane as a new tissue engineering tool

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Abstract: Recombinant Human Parathyroid Hormone (rhPTH 1–34) administration is an effective treatment to improve bone mass in osteoporosis. The aim of this study was to develop a Tissue Engeenering Tool for bone regeneration. We evaluated the efficacy of a freeze dried rhPTH membrane in calvarial critical size defect (CSD). Forty-four Wistar female rats (body weight 150 ± 50 g) with CSD (5 mm) were divided into four groups: group 1: rhPTH membrane (rhPTHm); group 2: atelocollagen membrane (Cm); group 3: rhPTH and atelocollagen I (CrhPTHm); group 4: without any treatment (CG). All samples were evaluated on the 1st, 3rd, and 6th weeks (weeks) postsurgery by soft X-ray, histological and histometric studies. Soft X-ray results showed a radiolucent image with many irregular radiopaque areas. Histologically, rhPTHm was replaced by reticular bone (7%) since 3rd week, and lamellar bone ossicles (30%) at 6th week. Cm showed bone formation like composite bone type on week 1st, 3rd, and 6th (2%, 44%, and 41%, respectively). With CrhPTHm, bone formation was observed in all periods (2.4%, 48%, and 53%), showing statistical difference with CG in the 3rd and 6th wks ($p = 0.03$ and 0.01). Our results demonstrated the effectiveness of a new biomaterial called CrhPTHm because its ability to regenerate calvarial CSD. Moreover, the membrane represents a new local intermittent delivery system allowing rhPTH slow release. © 2014 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 00A:000–000, 2014.

Key Words: bone regeneration, critical size defect, rhPTH, type I atelocollagen, grafts

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INTRODUCTION

The use of grafts is a predictable and effective method of enhancing bone healing.[1] The only remaining matter of controversy is the choice of suitable bone replacement materials. Expanded polytetrafluorethylene membranes are commonly used, but they require a second surgical stage. To avoid this problem, resorbable polymer membranes seem to be an attractive alternative.[2] Collagen membranes have also been used because this protein plays a regulatory role in osteoblastic growth and differentiation.[3] Moreover, collagen is biocompatible, easily degradable, resorbable, and capable of interacting with biological systems, allowing cell adhesion, and proliferation.[4] It is extracted industrially from bovine and porcine dermis and tendons and it has multiple applications in the construction of biomaterials.

In 2002, Recombinant Human Parathyroid Hormone (rhPTH 1–34) was approved by the US Food and Drug Administration (FDA) for osteoporosis treatment.[5] From then on its effects were studied on other bone diseases of different etiology. To reproduce these circumstances, rhPTH administration was studied in different animal bone defect models. rhPTH promoted the synthesis of collagen type II in femoral and mandibular fractures and accelerated the natural healing process by shrinking callus size at early stages.[6–8] We demonstrated that rhPTH was able to stimulate new bone showing non proliferative forms of bone hyperostosis in calvarial critical size defect (CSD).[9] On the other hand, when rhPTH was used systemically to a local treatment, Kamo et al.[10] demonstrated that intermittent weekly administration of human parathyroid hormone (1–34) improved hydroxyapatite block bonding in ovariectomized rats. Andreassen and Cacciafesta[11] investigated how systemic PTH treatment influenced guided bone regeneration using a critical size calvarial defect covered with osteoconductive membranes. Yun et al.[12] also evaluated bone formation in calvarial defect treated locally with synthetic β tricalcium phosphate following systemic PTH administration. Both authors found that bone deposition was higher and also showed better mechanical strength. With respect to local delivery of PTH in the defect area, there are few works

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TABLE I. New Bone Volume

Week	1st	3rd	6th
Control group	0.14% ± 0.1	0.4% ± 0.2	1.06% ± 0.2
rhPTHm group	2% ± 0.1	7% ± 0.2	30% ± 3.9*
Cm group	2% ± 0.07	40% ± 1.3*	41% ± 0.6*
CrhPTHm group	2.4% ± 0.2	48% ± 2.7*	53% ± 0.9*

Mean and Standard Deviation Values at 1st, 3rd, and 6th weeks from Control, rhPTHm, Cm, and CrhPTHm Groups. New bone volume was expressed in %, $*p < 0.05$, Kruskal Wallis test.

focusing on the stimulation of cellular activity and regulation of tissue regeneration. Wei et al.[13] incorporated PTH into poly lactic-*co*-glycolic acid microspheres to control the release and bioactivity of the protein. Backstrom et al.[14] investigated PTH administered locally via a direct gene delivery which was found to be beneficial in the treatment of bone loss. Jung et al.[15] demonstrated that an arginine-glycine-aspartic acid modified polyethylene glycol-based matrix containing covalently bound peptides of PTH 1–34 enhanced bone regeneration in a mandibular bone defect. Up to now the combination of type I atelocollagen with recombinant human parathyroid hormone 1–34 has not been described as a local delivery system for bone loss regeneration. The aim of this study was to develop a Tissue Engeenering Tool for bone regeneration. We evaluated the efficacy of a freeze dried rhPTH membrane in calvarial critical size defect regeneration.

MATERIALS AND METHODS

Preparation of the membranes

For manufacture of membranes, 1 mL (3mg) of atellocolagen type I (Collagen Solution, 3 mg/mL ultra Pure, SIGMA), 0.1 mL (20 μg) of rhPTH (Teriparatide, Forteo 3 mL, Eli Lilly) or a mixture of both was placed in sterile tubes (one for each sample) and lyophilized.[16]

Animals

Forty-three female *Rattus norvegicus* var. Wistar rats (150 ± 50 g), ~9 weeks old, were obtained from the Animal Research Center (Medical School, Tucumán University, Argentina). Animals were housed in pairs, in a specific pathogen-free environment, with a temperature of 22.4°C–23.8°C, relative humidity of 45–62%, and a 12-h light-dark cycle. A standard commercial diet and tap water were available *ad libitum*. During the study, animals were handled in accordance with the *Guide for the Care and Use of Laboratory Animals*, *8th Edition* (NRC, 2001) from the National Academic Press (Washington DC, USA).

Experimental design

The research protocol was approved by the local Ethical Committee for Animal Research (Tucumán National University & CONICET). Critical size defects were created in calvariae as previously described.[17,18] Briefly, defects were created manually with 5-mm-diameter *ad hoc* punch with smooth edges. Animals were randomly assigned to one of four groups. Group 1: treated with rhPTH membrane (rhPTHm); group 2: treated with atelocollagen membrane (Cm); group 3: treated with atelocollagen and rhPTH membrane (CrhPTHm); group 4: without treatment. After 1, 3, or 6 weeks postsurgery, animals were killed. Bone samples were obtained and fixed in 20% buffered formalin phosphate for 24 h.

Soft X ray—High-resolution films

All samples were subjected to soft X-ray with high resolution Kodak film (mammography type, 18 × 23 cm; GBA Mamograf HF Digital brand equipment, Buenos Aires, Argentina). Exposure time was 0.8 s, at 27.5 kV and 7.0 mA.[19]

Histology sample preparation

The specimens were decalcified with modified Morse solution (Okayama University Dental School) and embedded in paraffin in a routine manner. Two serial 4 μm central sections per defect were selected and stained with Hematoxylin & Eosin (H&E) and Masson's Trichrome. They were examined by light microscopy. A single pathologist evaluated all tissues. Subsequently, another pathologist (certified by the Argentina Health Ministry N° 31455) performed an independent review to verify microscopic observations. The reported results reflect the mutually agreed-upon diagnoses by both pathologists.

Histometric studies

Photomicrographs were taken from slides of each specimen by means of a Sony digital camera adapted to an Olympus CH30 microscope. The photos obtained by Soft Pinnacle Studio 9.4 with 116.7× magnification were evaluated by Image Pro Plus analysis system (Media Cybernetics, Silver Spring, MD, USA Version 4.5.0.29 for Windows 1998/NT/2000). New bone formation was quantified at the CZD area and expressed in percentage.

Statistical analysis

Data are presented as mean ± standard deviation, as indicated in Table I. Morphometric results were analysed by the Kruskal Wallis test ($p < 0.05$).

RESULTS

Soft X ray—High resolution films

At the 1st week postsurgery, soft X-ray images of CSD from all treated groups showed diffuse radiopacity of similar size, covering almost the entire defect. It was related to the implanted membrane presence. In the treated groups, at 3rd and 6th weeks, an increased diffuse radiopacity, with irregular spots, at center and edges of the CSD were observed. These areas wereconfirmed by histological study as new bone formed. In the untreated group, a radiolucent area corresponding to the defect site was observed in all study period [Fig. 1(a–l)].

Histopathology and histometric observations

All animals survived the surgical procedures and were available for evaluation. No signs of infection were registered during the healing period or at the time of retrieval.

In the control group, during the 1st week postsurgery, the defect area exhibited granulation tissue and congestion

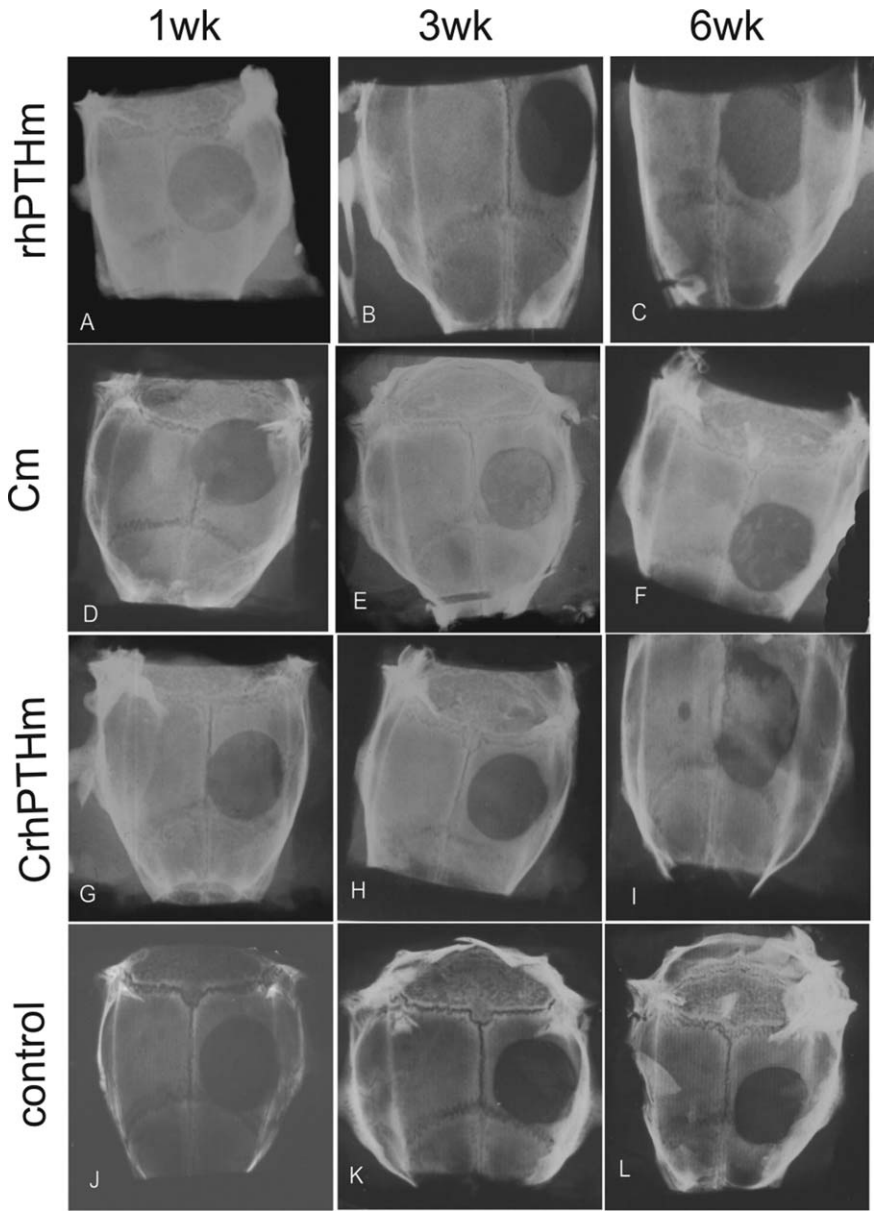

FIGURE 1. High resolution soft X-ray images in the 1st, 3rd, and 6th weeks showing 5 mm critical-sized defect diameter. Amorphous opacities can be seen in CSD. (a–c) rhPTHm, (d–f) Cm, (g–i) CrhPTHm, and (j–l) control groups. Magnification ×30.

in the superior sagittal sinus. In the 3rd and 6th weeks, the CSD area showed fibrous connective tissue healing. In all periods, there was almost no regenerated bone in the defects except in the immediate vicinity of the surgical margins. The amount of bone was 0.1 % ±0.1 at 1st week, 0.4 ± 0.2 at 3rd week and 1.06% ± 0.2 at 6th week.

In the rhPTHm group, during the 1st week, the CSD area showed granulation tissue and the rhPTH membrane appeared associated with fibrino-hemorrhagic exudates and colonized by mesenchymal-like cells [Fig. 2(a)]. During the 3rd week, there was a fibroblastic tissue, and the implanted membrane was partially replaced by woven bone [Figs. 2 (b,c)]. During the 6th week, lamellar bone ossicles were observed [Fig. 2(d)]. The morphometric results showed at 1st week 2% ± 0.1, at 3rd week 7% ± 0.2 and at 6th weeks 30% ±3.9.

In the Cm group, in the 1st week, the CSD area showed granulation tissue and congestion of the upper sagittal sinus. Type I atelocollagen membrane was observed covering the defect, surrounded by many mesenchymal cells. Below it there were some woven bone ossicles [Figs. 3(a,b)]. In the 3rd week, Cm was still detected with bone formation. The bone formed resembled composite bone type, characterized by lamellar cortical bone formation around vessels within a cancellous bone stroma[17] [Fig. 3(c)]. During 6th week, almost total replacement of collagen membrane by woven bone tissue with mineralized areas was observed [Fig. 3(d)]. The amount of new bone obtained

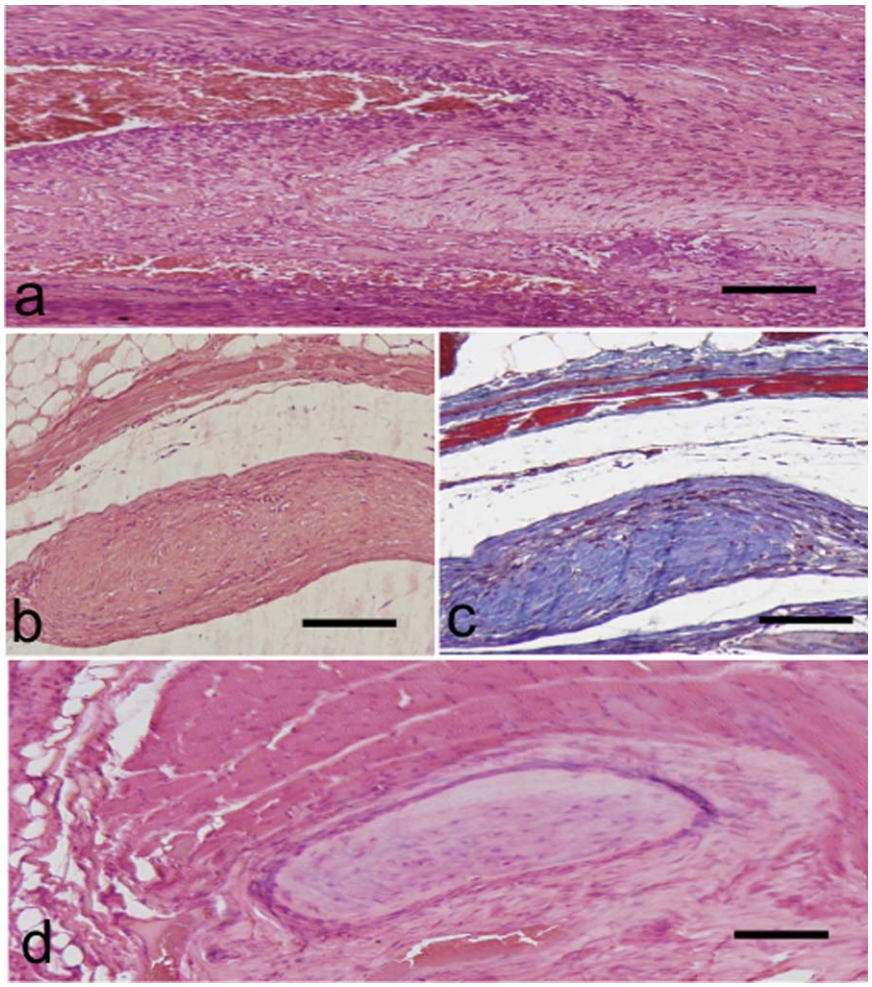

FIGURE 2. Histological analysis of CSD treated with rhPTHm. (a) H&E stained sections of rhPTHm associated with fibrino-hemorrhagic exudates and colonized by mesenchymal-like cells in the 1st week. (b) H&E and (c) Masson's Trichrome stained sections of the implanted membrane replaced by reticular bone in the 3rd week. (d) H&E stained sections of lamellar bone ossicles on the defect area in the 6th week. Magnification $\times$120, bar = 200 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

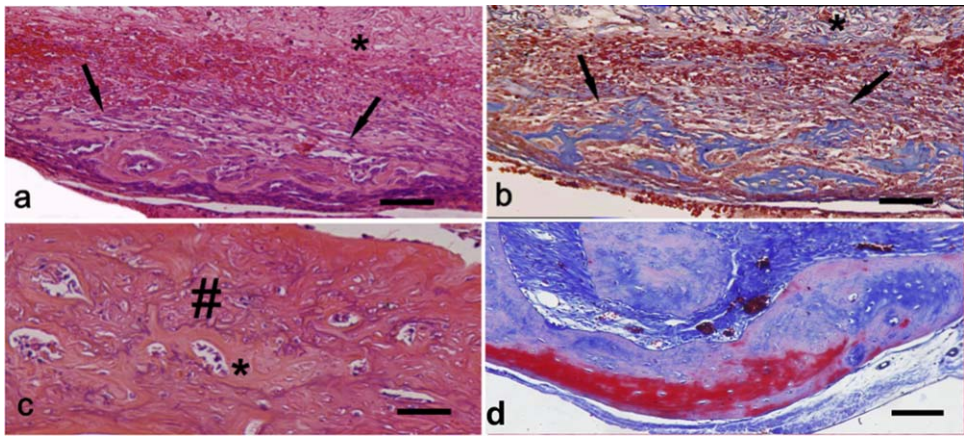

FIGURE 3. Histological analysis of critical-sized defect treated with Cm. (a) H&E- and (b) Masson's Trichrome stained sections of Cm were distinguishable covering the defect surrounded by mesenchymal cell proliferation (*). Below it there were woven bone ossicles (arrows) in the 1st week. (c) H&E stained sections of composite bone type characterized by lamellar cortical bone formation around vessels (*) within a cancellous bone stroma (#) in the 3rd week. (d) Masson's Trichrome stained sections of total replacement of Cm by woven bone tissue with mineralized areas in the 6th week. Magnification $\times$120, bar = 200 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

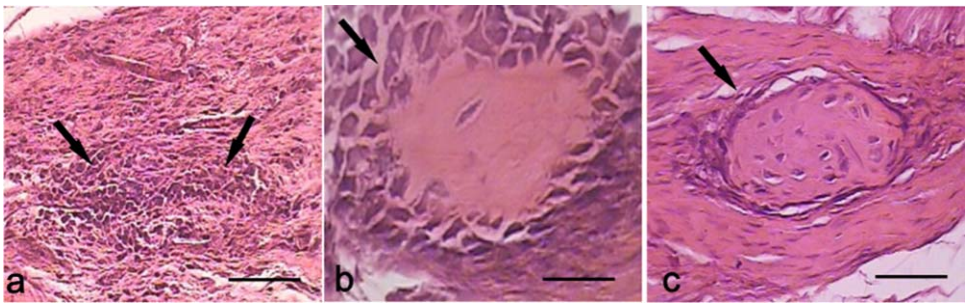

FIGURE 4. (a) H&E stained section of cube shaped cells arranged along the CrhPTHm resembling osteoprogenitor cells in the1st week. (b) and (c) H&E stained sections of lamellar bone islands in the 6th week. Magnification 466, ×8, bar = 250 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

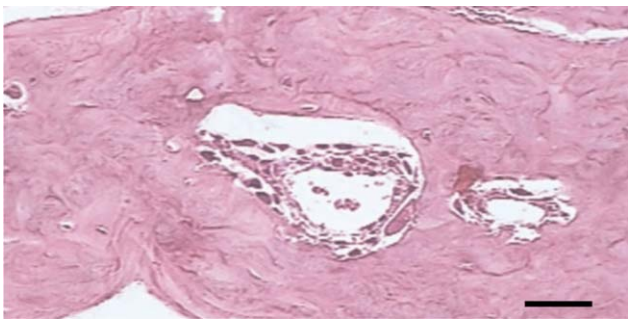

FIGURE 5. H&E stained section of new bone formation in CrhPTHm with a random precipitation of extracellular matrix in the 6th week. Magnification ×120, bar = 200 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

with this treatment was 2% ± 0.07 at 1st week, 40% ± 1.3 at 3rd week and at 6th week 41% ±0.6.

In the CrhPTHm, in the 1st week, the CSD area showed granulation tissue and congestion of the upper sagittal sinus, similar to control group. The biomaterial was covering the defect area and was seeded by osteoprogenitor like cells [Figs. 4(a)]. Small islands of woven bone were observed. During the 3rd week many nest of osteoprogenitor cells were placed along the membrane [Fig. 4(b)]. Bone islands were larger than those observed in the 1rd week [Fig. 4(b,c)]. In the 6th week, the membrane was transformed in laminar bone with a random mineral precipitation (Fig. 5). The histometrical results showed at 1st week 2.4% ± 0.2, at 3rd week 48% ± 2.7 and at 6th week 53% ± 0.9.

Figures 6(a,b) shows the defect on calvaria in treated and control groups at 6th weeks.

A bar graph shows the percentage of new bone formation in the four groups (Fig. 7). The table summarizes the histometrical analysis. Data were subjected to Kruskal Wallis Test. A statistically significant difference was observed in the rhPTHm group during the 6th week ($p = 0.01$), in the Cm group on the 3rd and 6th weeks ($p = 0.03$ and 0.01, respectively) and in the CrhPTHm group on the 3rd and 6th weeks (0.03 and 0.01, respectively).

DISCUSSION

This study demonstrated that the combination of rhPTH and atellocolagen type I significantly increase the amount of bone regeneration on critical size bone defect.

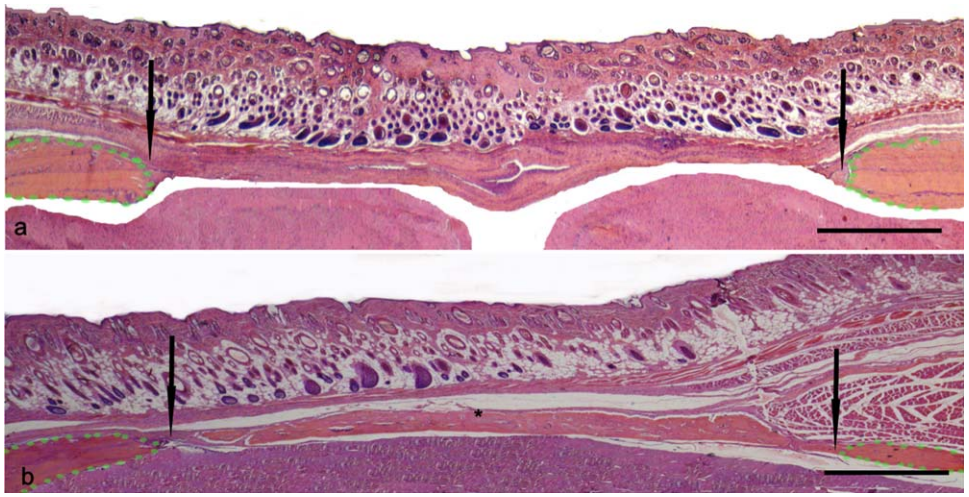

FIGURE 6. Histological analysis of CSD in the 6th week at low magnification (×67, bar = 2 mm) on H&E-stained sections. The defect margins are indicated by green dashes. (a) Control group: the defect was not regenerated except in the immediate vicinity of the surgical margins (arrows). (b) CrhPTHm group: defect regeneration was almost complete (arrows) (*). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

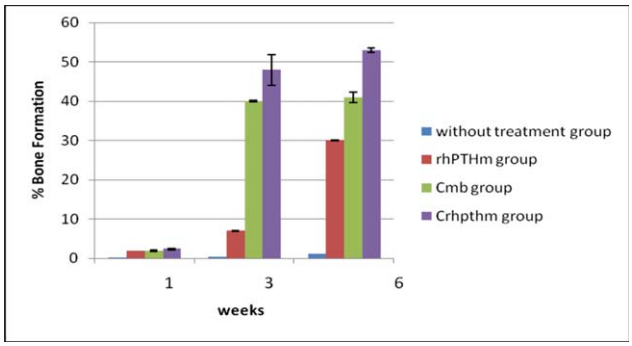

FIGURE 7. A bar graph shows the percentage of new bone formation in the four groups. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

In our experience, calvaria critical bone defect is a suitable experimental model to study bone regeneration, associated to fibrogenesis rather than osteogenesis when stimulation by replacement bone materials were absence. This was demonstrated in previous studies.[9,18–20]

Teriparatide has been shown to considerably increase cancellous and cortical bone mass,[21–23] improve bone microstructure and prevent fractures when administered subcutaneously at a daily dose of 20 μg to patients with osteoporosis.[24] Intermittent recombinant human parathyroid hormone 1–34 is used to treat severe osteoporosis in post menopausal women because of its anabolic effect.[5]

We hipotetized that rhPTH locally applied, could be an appropiate biomaterial able to regenerate bone defects. We manufactured a freeze dried membrane containing rhPTH alone or combined with type I atelocollagen.[16] rhPTH dose was selected based on previous studies[5,21–24] that evaluated rhPTH biological effects on bone tissue in animals and human. In rats an increase in bone mass without side–effects was observed when 5 or 10 μg/kg/day were used.[21–23] Moreover, we demonstrated that 20 μg/kg/day of rhPTH for systemic administration on rat calvarial critical-sized defect, increased bone formation, without side effects.[9]

Actually is well known that intermittent rather than continuous administration of PTH enhanced bone regeneration.[5,11,12] In this regard, the use of Type I atelocollagen as a vehicle for rhPTH was another interesting aspect from our findings. The dose was selected based on previous research from our team, where atelocollagen was an excellent vehicle, in order to liberate BMP protein, into the surrounding tissue to stimulate bone cells differentiation and proliferation.[16] It acted as a scaffold that allowed rhPTH slow release simulating a local intermittent delivery administration.[16] Furthermore, the collagen was incorporated on bone matrix without degradation.

The membrane obtained was placed in the CSD and then evaluated by histological and histometrical studies.

CSD area histological analyses showed CrhPTHm promising results, because the membrane induced cell proliferation and it was transformed into woven bone at 1th week, which appeared organized into lamellar ossicles covering the defect, at 6th week. It is important to note that we did not found researches about this form of rhPTH local application.

In agreement with our results, recently was reported bone healing at non critical osteochondral defect into rabbit knee, when a parathyroid hormone related protein (PTHrp) was injected into a collagen silk scaffold each 7 days.[25] But, unlike us, the intermittent effect of the peptide was obtained by subsequently PTHrp injection in the treated bone defect in different times; also we consider that our combined membrane resolved the issue in a suitable manner.

The amount of bone obtained from our histometric studies, at 6 weeks revealed the highest percentage (53% $\pm$0.9) when CrhPTHm was applied. This situation would be explained by synergistic effect between both proteins when they are applied locally as a membrane. Even that the underlying mechanism here proposed, was previously mentioned by other researchers.[25,26] Importantly, Zhang et al.[25] evaluated a bi layer collagen silk scaffold mixed with hydroxiapatite and rhPTH was injected as a systemic co adjuvant and not locally. About Jung et al.[26] bone augmentation was observed when a synthetic matrix containing PTH combined with hydroxiapatite was applied. So they differ from us, because in these circumstances, bone formation was mainly obtained by the placement of a bone graft like the hydroxyapatite and not exclusively to the hormone.

In summary rhPTH local application as a membrane was effective to stimulated bone regeneration at calvarial critical size defects. Furthermore, its combination with type I atelocollagen represents a new local intermittent delivery system allowing rhPTH slow release.

Our study provides valuable information for a future tissue engineering tool and its clinical translation for bone defect repair.

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REFERENCES

1. Dahlin C, Linde A, Gottlow J, Nyman S. Healing of bone defects by guided tissue regeneration. Plat Reconstr Surg 1988;81:672–676.
2. Dupoirieux L, Pourquier D, Picot M, Neves M. Comparative study of three different membranes for guided bone regeneration of rat cranial defects. Int J Oral Maxillofac Surg 2001;30:58–62.
3. Shi S, Kirk M, Kahn A. The role of type I collagen in the regulation of osteoblast phenotype. J Bone Mineral Res 1996;11:735–742.
4. Rodriguez A, Missana L, Nagatsuka H, Gunduz M, Tsujigiwa H, Rivera R, Nagai N. Efficacy of atelocollagen honeycomb scaffold in bone formation using KUSA/A1 cells. J Biomed Mater Res A 2006;77:707–717.
5. Hodsman A, Bauer D, Dempster D, Dian L, Harris S, Kendler D, McClung M, Miller P, Olszynski W, Orwoll E, Yuen CK. Parathyroid hormone and teriparatide for the treatment of osteoporosis: A review of the evidence and suggested guidelines for its use. Endocr Rev 2005;26:688–703.
6. Nakazawa T, Nakajima A, Shiomi K, Moriya H, Einhorn T, Yamazaki M. Effects of low dose, intermittent treatment with recombinant human parathyroid hormone (1- 34) on chondrogenesis in a model of experimental fractures healing. Bone 2005;37: 711–719.

7. Manabe T, Mori S, Mashiba T, Kaji Y, Iwata K, Komatsubara S, Seki A, Sun Y, Yamamoto T. Human parathyroid hormone (1–34) accelerates natural fracture healing process in the femoral osteotomy model of cynomolgus monkeys. Bone 2007;40:1475–1482.
8. Rowshan H, Parham M, Baur D, McEntee R, Cauley E, Carriere D, Wood J, Demsar W, Pizarro J. Effect of intermittent systemic administration of recombinant parathyroid hormone (1–34) on mandibular fracture healing in rats.J Oral Maxillofac Surg. 2010; 68:260–267.
9. Jammal MV, Pastorino N, Abate C Missana L. Bone-healing pattern on critical-sized defects treated by rhPTH. J Hard Tissue Biol 2012;4:443–450.
10. Kamo K, Miyakoshi N, Kasukawa Y, Nozaka K, Sasaki H, Shimada Y. Intermittent weekly administration of human parathyroid hormone (1–34) improves bone-hydroxyapatite block bonding in ovariectomized rats. J Bone Miner Metab 2010;28:634–640.
11. Andreassen T, Cacciafesta V. Intermittent parathyroid hormone treatment enhances guided bone regeneration in rat calvarial bone defects. J Craniofacial Surg 2004;15:424–427.
12. Yun J, Wikesjö U, Borke J, Bisch F, Lewis J, Herold R, Swiec G, Wood J, McPherson J III. Effect of systemic parathyroid hormone (1–34) and a beta-tricalcium phosphate biomaterial on local bone formation in a critical-size rat calvarial defect model. J Clin Periodontol 2010;37:419–426.
13. Wei G, Pettway G, McCauley L, Ma P. The release profiles and bioactivity of parathyroid hormone from poly (lactic-co-glycolic acid) microspheres. Biomaterials 2004;25:345–352.
14. Backstrom K, Bertone A, Wisner E, Weisbrode S. Response of induced bone defects in horses to collagen matrix containing the human parathyroid hormone gene. Am J Vet Res 2004;9:1223–1232.
15. Jung R, Cochran D, Domken O, Seibl R, Jones A, Buser D, Hammerle C. The effect of matrix bound parathyroid hormone on bone regeneration. Clin Oral Implants Res 2007;18:319–325.
16. Missana L, Nagai N, Kuboki Y. Comparative histological studies of bone and cartilage formations induced by various BMP-carriers composites. Jpn J Oral Biol 1994;36:9–19.
17. Roberts W. Fundamental principles of bone physiology, metabolism and loading. In: Naert I, Van Steenberghe D, Worthington P, editors. Osseointegration in Oral Rehabilitation. Carol Stream, IL: Quintessence Publishing; 1993. p 157–170.
18. Schmitz JP, Hollinger JO. The critical size defect as an experimental model for craniomandibular nonunions. Clin Orthopaedics Related Res 1986;205:299–308.
19. Aybar A, Territoriale E, Missana L. Animal model in calvaria to evaluate bone strategies. Acta Odontológica Latinoamericana 2005;18:21–25.
20. Jammal M, Territoriale E, Abate C, Missana L. High resolution films for bone regeneration evaluation. Acta Odontológica Latinoamericana 2010;23:33–36.
21. Vahle J, Sato M, Long G, Young J, Francis P, Engelhardt J, Westmore M, Linda Y and Nold J. Skeletal changes in rats given daily subcutaneous injections of recombinant human parathyroid hormone (1–34) for 2 years and relevance to human safety. Toxicologic Pathol 2002;30:312–321.
22. Vahle J, Long G, Sandusky G, Westmore M, Ma L, Sato M. Bone neoplasms in F344 rats given teriparatide (rhPTH 1–34) are dependent on duration of treatment and dose. Toxicologic Pathol 2004;32:426–438.
23. Jollette J, Wilker C, Smith S, Doyle N, Hardisty J, Metcalfe A, Marriott T, Fox J, Wells D. Defining a noncarcinogenic dose of recombinant human parathyroid hormone 1–84 in a 2-year study in Fischer 344 rats. Toxicologic Pathol 2006;34:929–940.
24. Whitfield JF, Morley P, Willick GE. Parathyroid hormone, its fragments and their analogs for the treatment of osteoporosis. Treat Endocrinol 2002;1:175–190.
25. Zhang W, Chen J, Tao J, Hu C, Chen L, Zhao H, Xu G, Heng BC, Ouyang HW. The promotion of osteochondral repair by combined intra-articular injection of parathyroid hormone-related protein and implantation of a bi-layer collagen-silk scaffold. Biomaterials 2013;25:6046–6057.
26. Jung R, Hämmerle C, Kokovic V, Weber F. Bone regeneration using a synthetic matrix containing a parathyroid hormone peptide combined with a grafting material. Int J Oral Maxillofac Implants 2007;22:258–266.