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Vertical Alveolar Ridge Regeneration by Means of Periosteal Activation—A Proof-of-Principle Study

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ABSTRACT

Aim: To assess the possibility of vertical alveolar ridge augmentation by means of activation of the periosteum.

Materials and Methods: Six adult male Beagle dogs were used for the study. All premolars and first molars were extracted, and one vertical saucer-shaped bony defect was created on each side of the mandible. After 3 months of healing, full-thickness muco-periosteal flaps were elevated, and one distraction device was placed on each side of the mandible. The distraction plate was left submerged, and the activation mechanism connected to the distraction rod was exposed intra-orally. The protocol of periosteal activation (PP: periosteal 'pumping') was initiated after a latency of 7 days. The alternation of activation and relaxation at the rate of 0.35 mm/12 h during 5 days was followed by the sole activation of 0.35 mm/12 h for 5 days (PP group). Devices were left inactivated on the contralateral control side of the mandible (C group). All animals were euthanized after 8 weeks of consolidation. Samples were analysed histologically and by means of micro-CT.

Results: New mature lamellar bone was formed over the pristine bone in all groups. More intensive signs of bone modelling and remodelling were observed in the PP group compared to the C group. Mean new bone, bone marrow, connective tissue and total volumetric densities were greater in the PP group ($p < 0.001$, $p = 0.001$, $p = 0.003$ and $p < 0.001$, respectively). No differences were observed in the relative area parameters. Total tissue volume and bone volume were higher in the PP group ($p = 0.031$ and $p = 0.076$, respectively), while the bone mineral densities were higher in the C group ($p = 0.041$ and $p = 0.003$, respectively). Trabecular number, trabecular thickness and trabecular separation values were similar between the two groups.

Conclusions: Regeneration of vertical alveolar bone ridge defects may be enhanced by activation of the periosteum, without the application of bone grafting materials.

1 | Introduction

Regeneration of vertical alveolar bone deficiencies is considered the most challenging procedure in the field of pre-prosthetic implant surgery (Urban et al. 2019). Initially, autogenous cortico-cancellous bone block grafts were proposed for vertical ridge augmentation (Triplett and Schow 1996). The autogenous bone block grafts were considered as a 'gold standard' for augmentation of extended bone deficiencies due to their osteoconductivity and presumptive osteoinductivity (Miron et al. 2011).

The main advantage of the autogenous bone grafts is the presence of viable cells, which provide a favourable paracrine micro environment (Miron et al. 2013). Cortical bone is known for a low resorption rate and a progressive, complete revascularization and remodelling of a non-vital bone (Acocella et al. 2010), exerting sufficient stability (Smolka et al. 2006). The replacement of a non-vital bone by a vital bone during graft remodelling proceeds through an inflammatory and vascular response from the surrounding soft tissues (Aalam and Nowzari 2007). However, the most common complication in autogenous bone block grafting was reported to be a fast and unpredictable rate of initial bone resorption (Clementini et al. 2012; Roccuzzo et al. 2007). Furthermore, the exposure of the surgical site following a perforation of the mucosa has been reported frequently (Urban, Montero, Amerio et al. 2023). In view of the apparent shortcomings including donor-site morbidity (Clavero and Lundgren 2003; Cordaro, Amade, and Cordaro 2002), the assessment of presumptive alternative augmentation techniques was considered desirable.

Guided bone regeneration (GBR) is the standard of care for augmentation of missing bone prior to or simultaneously to implant placement (Lang et al. 1994). Vertical GBR was found to enable the formation of considerable amounts of biological structures mimicking native tissues (Urban, Montero, Amerio et al. 2023). In fact, vertical GBR using non-resorbable titanium membranes was ranked superior to other surgical techniques in terms of vertical bone gain (Alotaibi et al. 2023). The survival rate of implants is high, but the utilization of at least 50% of autogenous bone chips appears to be indicated in most instances. Nonetheless, the rigid membranes may block the access to the osteogenic cells derived from the periosteum (Simion et al. 2006). Exposure of the membrane and the subsequent site infection remains the main concern in vertical GBR (Fontana et al. 2011).

Vertical alveolar ridge augmentation techniques with increased vertical bone gain were associated with increased incidence of complications (Alotaibi et al. 2023). Soft-tissue management and suturing techniques for different vertical augmentation techniques, however, follow the same principles (Urban, Montero, Sanz-Sanchez et al. 2023). For flap advancement and the primary closure of the wound, a periosteal releasing incision, periosteoplasty or even a partial excision of periosteum may be indicated. Furthermore, periosteum elevated from the original bony surface does not contribute to the supraosteal bone formation (Kostopoulos and Karring 1994). The viability of periosteum is heavily impaired, leading to a delayed healing process. Hence, bone has to originate from the endosteum and marrow spaces of the pristine bone only (Dahlin et al. 1988). Since the regenerative capacity is generally limited and constrained by inflammation or disease, a promising alternative is to stimulate the inherent self-repair mechanisms to promote endogenous restoration of the lost bone (X. Wang et al. 2018).

Periosteum plays a crucial role in callus formation during fracture healing and distraction osteogenesis (DO) (Ai-Aql et al. 2008). The principle of DO is based on a progressive elongation of the bone fragments created through osteotomy, resulting in endogenous hard- and soft-tissue formation (Ilizarov 1989). DO was successfully applied in the treatment of extended vertical deficiencies of the alveolar ridges (Chiapasco et al. 2004), but the indications for DO are limited with regard to the type and the stage of alveolar ridge resorption (Saulacic, Zix, and Iizuka 2009). If the transport segment consists only of the cortical bone, reduced blood supply and increased likelihood of secondary resorption may be expected (Saulacic, Zix, and Iizuka 2009; Wolvius et al. 2007). More recently, it has been demonstrated that a gradual elevation of the periosteum from the original bony surface could promote a progressive soft-tissue adaptation and hard-tissue formation (Garcia-Gonzalez et al. 2021). Negative contributions of the elevated periosteum to the osteogenic process may thus be reversed by using protocols similar to those developed for conventional DO. In contrast to block bone harvesting or conventional DO, osteotomy is not performed. Immediately after elevation, a strong proliferative response in the periosteum occurs (Mouraret et al. 2014). This represents a stabilization of the blood clot, followed by the formation of a well-organized, dense cellular fibrous tissue, without evidence of an inflammatory infiltrate. Following the activation period, the distraction gap formed by periosteal distraction osteogenesis (PDO) is bordered by the original surface of the bone base and by the periosteal (i.e., cambial) layer. However, it remains unclear whether the manipulation of PDO parameters is crucial for complete bone fill.

Preliminary data have indicated that the protocol of periosteal activation through pumping-like movements (PP) by means of alternated activation and relaxation could enhance apposition of new bone (Saulacic, Vunjak-Novakovic et al. 2022). Furthermore, the inclusion of the PP protocol provided a more accentuated improvement in bone formation when compared to the standardized PDO in regeneration of extended lateral alveolar ridge deficiencies (Saulacic, Garcia-Gonzalez et al. 2022). Hence, the present study aimed to assess the potential of a PP protocol to prevent vertical alveolar ridge atrophy and enhance the intrinsic periosteal stimulation in the mandibles of dogs as a proof of principle. The null hypothesis was that of no difference in bone formation for the PP protocol compared to the device placement without activation. The primary outcome was the amount of newly formed bone assessed histomorphometrically.

2 | Materials and Methods

2.1 | Animals and Surgery

Six female Beagle dogs (age 19 months) were used. The animals were housed in a group kennel with indoor (temperature of 20°C–22°C with natural light and air renewal) and outdoor areas. During the entire study period, the dogs received a soft-food diet and water ad libitum. The study protocol followed the guidelines of the European Union Council Directive of 1 February 2013 (R.D.53/2013), approved by the Ethics Committee of the Rof Codina Foundation, Lugo, Spain (AELU001/66548/RX 1548438). In addition, the study complied with the Guidelines for Animal Research: Reporting In Vivo Experiments (ARRIVE) 2.0.

The animals were premedicated with morphine (0.4 mg/kg/im, Morfina Braun 2%; B. Braun Medical, Barcelona, Spain) and medetomidine (20 μg/kg/im; Esteve, Barcelona, Spain). The anaesthesia was induced by propofol (3–5 mg/kg/iv; Propovet, Abbott Laboratories, Kent, UK) and maintained by inhalation of an O_2 and 2.5%–4% isoflurane mixture (Isobavet, Schering-Plough, Madrid, Spain). Mandibular branch nerve blocks were performed with lidocaine and adrenalin (Anesvet, Ovejero, León, Spain). Prophylactic antibiotics were applied intra-operatively using cefovecin (8 mg/kg/sc, Convenia, Zoetis) and cefazolin (20 mg/kg/iv, Kurgan, Normon). Atipamezole (50 µg/kg/im; Esteve) was administered to revert the effects of medetomidine.

In the first surgery, all premolars and the first molars (PM1, PM2, PM3, PM4, M1) were extracted within a flapless procedure. One saucer-shaped bony defect measuring 20 mm in length and 10 mm in depth was created on each side of the mandible, and the wounds were closed with resorbable sutures (Vicryl 5-0, Ethicon, Issy Les Mou-lineaux Cedex, France). After 3 months of healing, a custom-made distraction device was placed on each side of the mandible. The mid-crestal incision was performed, and two full-thickness mucoperiosteal flaps were elevated on the buccal and lingual sites (Figure 1A,B). The basal plate of the internal part of the device was fixed to the pristine bone using two micro-screws (5 × 0.9 mm, Medartis AG, Basel, Switzerland) (Figure 1C). Minor reshaping of the bony defect contours was necessary at the bottom of the bony defect in 9 out of 12 sites (5 PP and 4 C groups, respectively) to allow the placement of the tip of distraction plate. The external part of the device was adapted to the pristine bone and fixed with four screws (10 × 1.5 mm, Medartis AG) (Figure 1D). The distraction rod of the internal

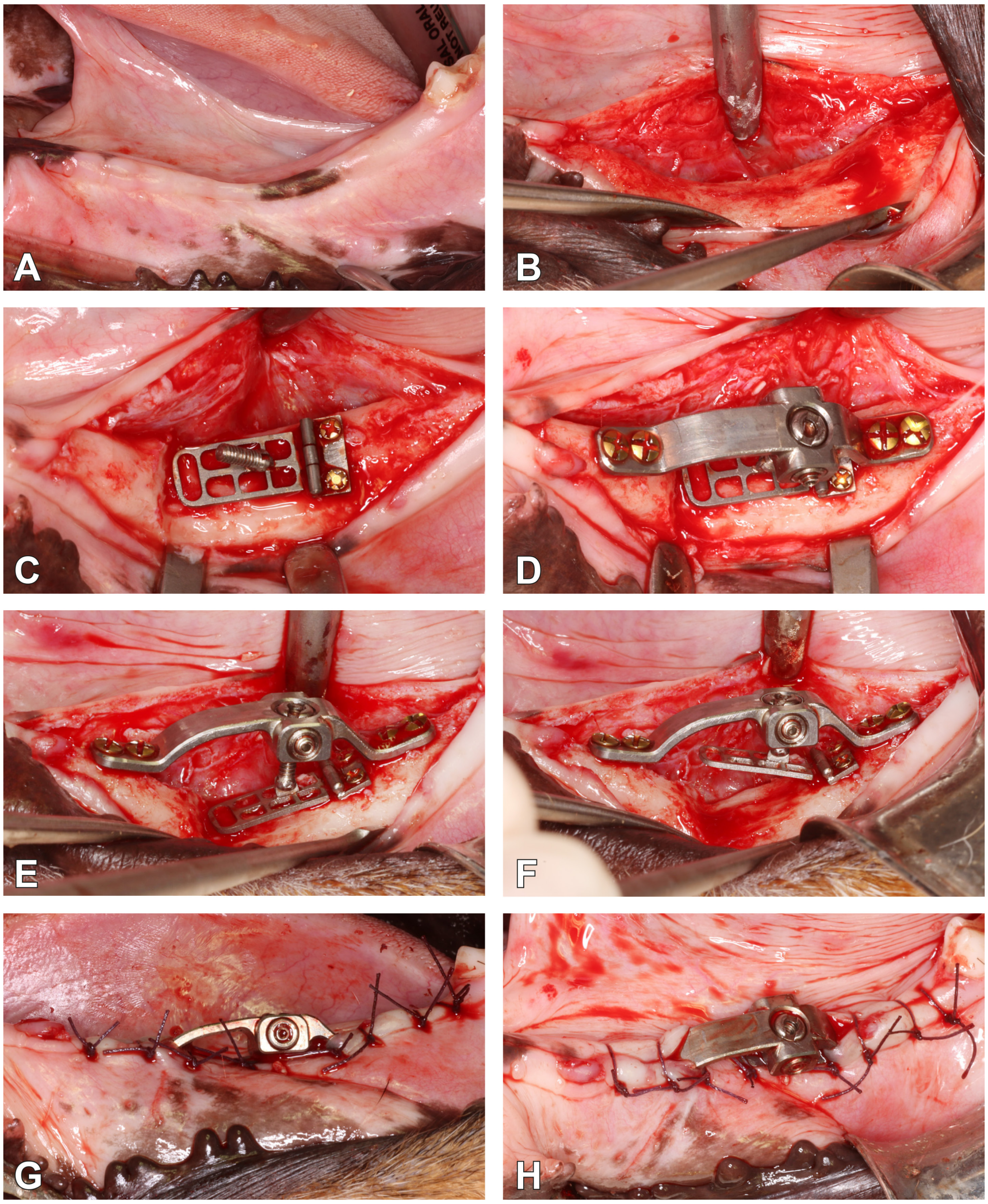

FIGURE 1 | Placement of the distraction devices during the second surgery. (A) Intra-operative view of the area 3 months after defect creation. (B) Two full-thickness mucoperiosteal flaps were elevated on the buccal and lingual side following crestal incision. (C) The basal plate of the device was fixed to the pristine bone using two micro-screws. (D) The external part of the device was fixed with four screws and the distraction rod was connected to the external element of the device. (E, F) The device was tested for functionality. (G, H) The wound was closed with interrupted sutures, leaving the distraction plate submerged and the activation mechanism exposed to the oral cavity.

part was connected to the activation mechanism of the external element, and tested for the functionality (Figure 1E,F). The wound was closed with resorbable sutures, leaving the internal part of the device submerged. The mid-part of external element including the activation mechanism was left exposed to the oral cavity (Figure 1G,H).

The animals were monitored daily for their health status during the entire procedure. Post-operative pain was controlled with morphine (0.3 mg/kg/im/6 h, Morfina Braun 2%, B. Braun Medical) for 24 h and meloxicam (0.1 mg/kg/s.i.d./po, Metacam, Boehringer Ingelheim, Barcelona, Spain) during the next 3 days. The teeth, oral mucosa and distractors were cleaned and disinfected three times a week using gauzes moistened in a 0.12% chlorhexidine solution (Perio-Aid Tratamiento, Dentaid, Barcelona, Spain). Afterwards, a toothbrush with 0.2% chlorhexidine gel (Chlorhexidine Bioadhesive Gel, Lacer, Barcelona, Spain) was used for plaque control three times a week.

The treatment modalities (with or without activation of the distraction device) were allocated according to a systematic randomizing protocol (www.randomization.com). Since both treatment modalities were performed on every dog, the unit of the analysis was the animal. Potential confounders were not specifically controlled. No sample size calculation was performed because of the exploratory nature of this study. The devices were activated on one side of the mandible after a latency of 7 days (PP group). The manipulation of the devices proceeded at the rate of 0.35 mm/12 h (Saulacic, Garcia-Gonzalez et al. 2022). An alternated protocol of activation and relaxation performed for 5 days was followed by a sole activation for 5 days until a total of 3.5 mm distraction had been achieved. Devices were not activated on the control side of the mandible (C Group). Calcein (12 mg/kg/im, B. Braun Medical) and alizarin red (30 mg/kg/im, B. Braun Medical) were administered on the first and the last day of distraction. Animals were euthanized after 8 weeks of consolidation.

2.2 | Euthanasia

Animals were sedated with butorphanol (0.1 mg/kg/im Butomidor, Richter Pharma, Austria) and medetomidine, induced by propofol (3–5 mg/kg/iv; Propovet, Abbott Laboratories) and euthanized with intravenous overdose of sodium pentobarbital (50–60 mg/kg/iv, Dolethal, Vetoquinol, France). Clinical examination was performed, and 12 segments with intact soft tissues were retrieved by sharp dissection and separated using a band saw. The samples were fixed in 10% buffered formaldehyde and proceeded to the micro-CT (μCT) and histological analysis.

2.3 | μCT Analysis

All sites were scanned using a high-resolution μCT system (SkyScan 1172, Bruker MicroCT NV, Kontich, Belgium). The x-ray source was set at 100 kV and 100 μA, with a pixel size of 13.57 μm. A filter of aluminium/copper was used. The scanning was performed over 360° rotation, obtaining images every 0.4°. The μCT slices (1000) were reconstructed perpendicularly to the sagittal axis of the alveolar ridge. Mineralized tissue was selected from grayscale images with a specific threshold of 50–255 in the volume of interest (VOI) excluding the distraction device to avoid measuring the plate as mineralized tissue. The obtained images were reconstructed using NRecon software (Bruker MicroCT NV) and evaluated using Data Viewer (Bruker MicroCT NV) and CtAn (Bruker MicroCT NV). The VOI was defined corresponding to the region of interest (ROI), and the new bone volume was defined according to trigonometry (Figure 2). Total tissue volume (TTV, mm^3), bone volume (BV, mm^3), bone mineral density (BMD, mg HA/mm^3), trabecular number (Tb.N), trabecular thickness (Tb.Th) and trabecular separation (Tb.Sp) were measured. Furthermore, the relative BV

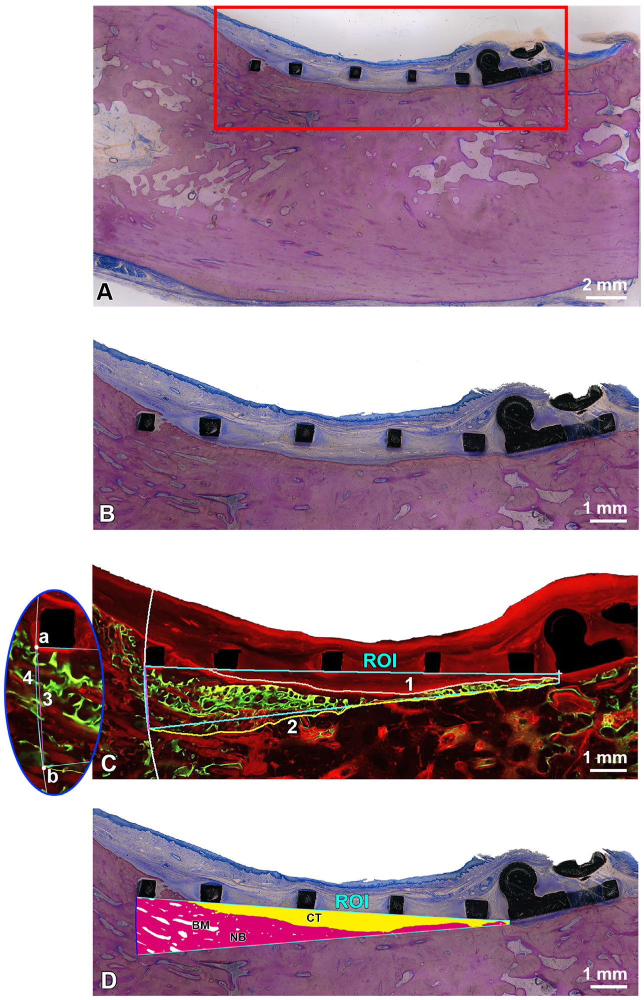

FIGURE 2 | Morphometric analysis. (A, B) The region of interest (ROI) was selected using a magnified image of the overview histological images. (C) Newly formed bone is delineated on the fluorescence image by two labelling lines of alizarin red (line 1) and calcein (line 2). The ROI was defined by a circle drawn from the base of the distraction plate and two straight lines corresponding to the inner side of the distraction plate and the calcein labelling. The cutting points a and b give rise to the line 3 used for the micro-CT evaluation, and the arc of circumference (line 4) corresponds to the distracted distance. (D) The obtained ROI on the fluorescence image (C) is transferred to the stained histological image (B). The area measurements (right) are coloured pink for new bone (NB), white for bone marrow (BM) and yellow for connective tissue (CT).

(%BV) was calculated as a ratio of the segmented BV to the TTV, and the relative BMD (%BMD) as a ratio of BV density to the TTV density.

2.4 | Histological Preparation and Analysis

Following fixation, samples were rinsed in running tap water, trimmed, dehydrated in ascending ethanol concentrations and embedded in methylmethacrylate. The embedded tissue blocks were cut in the mesio-distal plane into 1-mm thick sections using a slow-speed diamond saw (Varicut VC-50, Leco, Münich, Germany). After mounting on transparent acrylic glass slabs, the sections were ground and polished to a final thickness of 400 μm (Knuth-Rotor-3, Struers, Rødovre/Copenhagen, Denmark). Eight sections were prepared from each site: four sections from underneath, and four sections outside the distraction plate. The bone labelled by alizarin and calcein was digitally photographed under a confocal laser scanning microscope (LSM710; Carl Zeiss Microscopy GmbH, Jena, Germany). Surfaces were stained with basic fuchsin and toluidine blue/McNeal (Schenk, Olah, and Hermann 1984). Photographs were taken using a digital microscope (VHX 6000, Keyence, Tokyo, Japan).

Morphometric analysis was performed by a graphic software (Photoshop CS6; Adobe, San Jose, CA, USA, and Olympus CellSens Dimension 1.15, Olympus Corporation, Tokyo, Japan). The ROI was selected manually with the help of trigonometry, defined by the length of the distraction plate and two fluorescence lines (Figure 2). Total tissue area (TTA) was delineated by the inner side of the distraction plate, calcein labelling line and the arc of circumference corresponding to the distracted distance. The area fractions of the new bone area (NBA, mm^2), bone marrow area (BMA, mm^2) and connective tissue area (CTA, mm^2) were determined. Relative area parameters of the NBA (%NBA), BMA (%BMA) and CTA (%CTA) were measured as a percentage to the TTA. The morphometric parameters were quantified as previously described (Lang 2019). One examiner assessed the parameters of μCT and histomorphometry, without being aware of the samples' allocation.

2.5 | Statistical Analysis

The distribution was checked for normality, and variances were assessed using the Shapiro–Wilk test. The differences between two groups were compared using a Mann–Whitney *U* test. The analysis was done using the statistical software SigmaPlot 12.5 for Windows (Systat Software Inc., San Jose, CA, USA). A value of $p < 0.05$ was considered statistically significant.

3 | Results

3.1 | Clinical Observations

All animals recovered well from surgery. During the activation period, a fracture of the mandible occurred in one site (C group), and the distraction plate was exposed in one site (PP group) without noticeable infection. In none of the sites were the devices mobile, nor were the screws loosened. Clinical examinations revealed an uneventful healing in all other animals without complications related to the soft tissues. All animals had a normal behaviour without impairment on their general appearance or water and food intake.

3.2 | Histological Analysis

Signs of minor device exposure were detected on the histological sections in the hinge region of the internal part of the five surgical sites (two in the PP group and three in the C group). In general, minor locally confined inflammatory reactions were observed on the central sections above the distraction plate. All samples were included in the analysis ($n = 6$ per group). New bone formation was observed in all groups.

Generally, parallel-fibered lamellar bone with bony sprouts within the bone marrow was observed in all sites (Figure 3). Newly formed woven bone was seen facing the soft tissue in the PP group (Figure 3H,I). Osteoblasts and osteoid seams were detected at the apposition sites lining the bony surface and the bone marrow cavities. Signs of bone resorption and bone formation were observed at the bony surface. Osteoclasts and Howship's lacunae were detected at the resorption sites mainly in the C group, with signs of pristine bone remodelling (Figure 3F,L). New bone formation could still be visualized by calcein and alizarin-red fluorescence lines in both groups after the activation period (Figure 4). The treated sites were more intensively labelled in the PP group than in the C group, with the fluorochrome-labelled lines orientated parallel to the direction of distraction (Figure 4B,F,N).

In general, thickness of the new bone in the PP group exceeded that observed in the C group. There was no difference in the morphological pattern of bone formation underneath and outside the distraction plate in both groups. Accurate positioning of the reference line outside the distraction plate was, however, not possible. Consequently, the morphometric analysis was performed only underneath the distraction plate. The ROI was significantly greater in the PP group than in the C group ($p < 0.001$). All absolute area parameters were higher in the PP group than in the C group (Figure 5, Table S1), reaching statistically significant differences for NBA ($p < 0.001$), BMA ($p = 0.001$) and CTA ($p = 0.003$). The relative area parameters between two groups, however, did not reach statistical significance.

3.3 | μCT Analysis

μCT revealed homogenously distributed mineralized structures comprising old and new bone. Hence, newly formed bone could not be precisely distinguished from the old pristine bone because the material density was very similar. The PP protocol resulted in greater bone formation within the VOI when compared to the C group (Figure 6, Table S2). The PP group showed higher TTV and BV than the C group ($p = 0.031$ and $p = 0.076$, respectively), while both BMD and %BMD were significantly higher in the C group than in the PP group ($p = 0.041$ and $p = 0.003$,

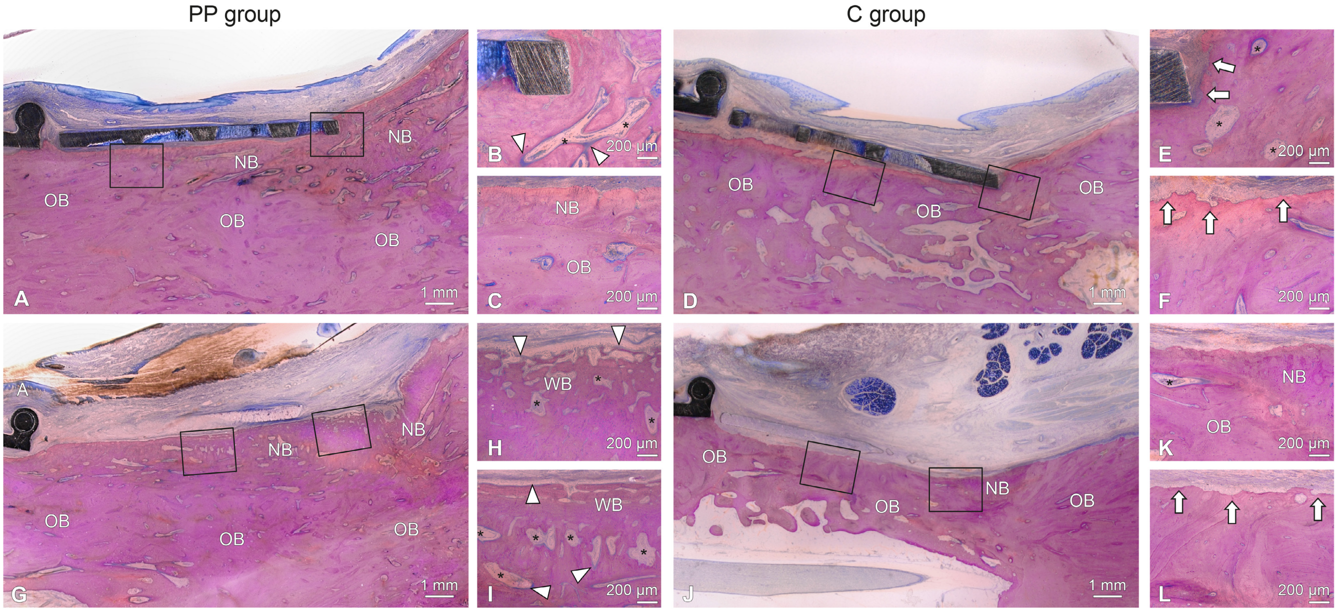

FIGURE 3 | Representative transversal histological images of the alveolar ridge illustrating sections in the mid-axis (A–F) and outside the distraction plate (G–L) in the PP and in the C group. Overviews showing new bone (NB) overlaying the old bone (OB). The thickness of the newly formed bone in the PP group is greater than in the C group. The boxed areas in the overviews left are magnified in the right frames. New bone in the PP group was mainly composed of the parallel-fibered and lamellar bone with intervening bone marrow cavities (*). Osteoid seams (arrowheads) were seen lining the surface of the woven bone (WB) and the bone marrow cavities. Irregular bony surface with Howship's lacunaes (arrows) were observed mainly in the C group, with signs of pristine bone remodelling.

respectively). Mean Tb.N, Tb.Th and Tb.Sp values did not differ between the two groups.

4 | Discussion

Vertical alveolar ridge augmentation is a complex, technique-sensitive intervention requiring an optimal management of hard and soft tissues. Present findings indicate that the mechanical manipulation of periosteum may be successfully applied for the regeneration of vertical bone deficiencies. Hence, it supports the current concept that controlled, gradual elevation of the periosteum induces bone formation at the site of apposition from the existing bony surface.

Owing to the fact that vertical alveolar ridge augmentation demands high skills for soft-tissue management, the wound dehiscence in the periosteal distraction remains a risk for unfavourable wound healing. In contrast to the bone block grafting or vertical GBR techniques, an exposure of the distraction plate to the oral environment inevitably leads to treatment failure. Remarkably, in the present study, the minor soft-tissue dehiscences were observed at the region of the hinge and not at the tip of the distraction plate. A possible reason for this phenomenon was the localization of the hinge that enabled plaque retention and food impaction. Signs of local inflammation in the region of the distraction rod were frequently observed; however, the distraction seemed to be advantageous for the absence of major infection (Saulacic, Zix, and Iizuka 2009; Wolvius et al. 2007). An increase in blood flow from the periosteum during distraction, facilitated by angiogenesis, is essential in the healing process (Yasui et al. 1991). Furthermore, an alternated distraction protocol is beneficial when compared to the compression following the activation period (Mori et al. 2006). In the latter case, a collapse of the blood vessels and decreased vascularity result. As a prerequisite, the position of the external element of the distraction device requires the maintenance of meticulous oral hygiene performed regularly.

The new bone formed in all groups resembled the characteristics of pristine bone. This corresponds to previous results observed at the intra-oral and extra-oral sites (Oda, Kinoshita, and Ueda 2009; Saulacic et al. 2016; Yamauchi et al. 2013). A clinically relevant model had been used in the present study, but bone formation in both groups may have to be attributed to the favourable healing capacity of dogs. Furthermore, undermining of the periosteum during the surgical procedure as well as the adaptation of the bony defect edges in both groups could have stimulated new bone formation (Nobuto et al. 2005). The interaction between bone and the periosteum via flap elevation may potentially yield better regeneration potential compared to osteotomy alone in conventional DO. Activated osteoprogenitor cells within the periosteum resulted in a favourable osteogenic effect in vertical ridge augmentation procedures (Chen et al. 2020). Deletion of Sost was responsible for triggering the endogenous Wnt signalling, which correlated with the bone formation. The benefits of the PP protocol over the device have been documented for all morphometric parameters compared to the C group. This, in turn, means that the null hypothesis of the present study stands rejected. The significance of the space provided by the distraction plate has been previously described (Saulacic et al. 2016; Saulacic, Garcia-Gonzalez et al. 2022).

Despite the histological findings indicating the benefit of the PP protocol, the differences in the BV between the two groups

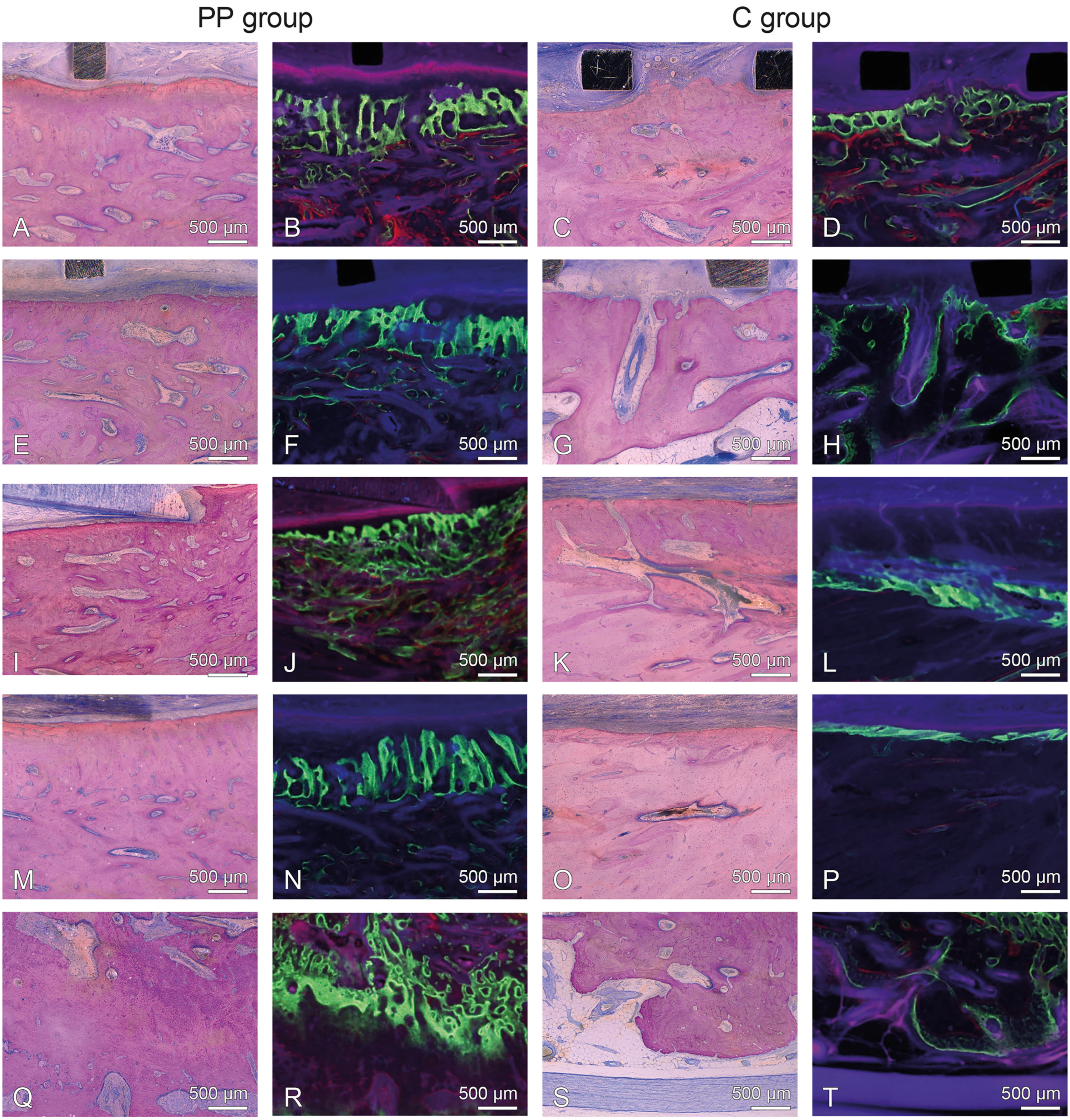

FIGURE 4 | Representative histological details of the treated sites with the labelled bone of the corresponding areas at the upper and lower part of the gap underneath (A–D and E–H, respectively) and outside the distraction plate (I–L and M–P, respectively), and next to the mandibular canal (Q–T) in the PP and C groups. The calcein-labelled regions (green) are recognizable in the new bone, covered with the alizarin-labelled regions (red). The fluorochrome-labelled lines in the PP group (B, F, N) are oriented parallel to the direction of distraction.

did not reach statistical significance. This may be due to the relatively small number of animals involved in the present study. Both BMD and %BMD were significantly higher in the C group. Nevertheless, relative values may be considered less accurate in reflecting the results than absolute values. Relative micro-CT and histomorphometric parameters were similar between the two groups, but both TTV and TTA were significantly lower in the C group than in the PP group. Minor amounts of newly formed bone in the C group were more rapidly mineralized, while the characteristics of bone formation were still observed in the PP group. Bone fluorochrome labelling revealed ongoing bone formation following the activation period of the device. Furthermore, the calcein fluorescence lines orientated along the vector of distraction were observed underneath and distally to the distraction plate, as well as on the sections outside the distraction plate. Bone formation outside the distraction plate may be accentuated by an undisturbed interaction between the periosteum and the underlying bone (Ai-Aql et al. 2008; Canalis and Burstein 1985; Hirashima et al. 2015), However, newly formed bone outside

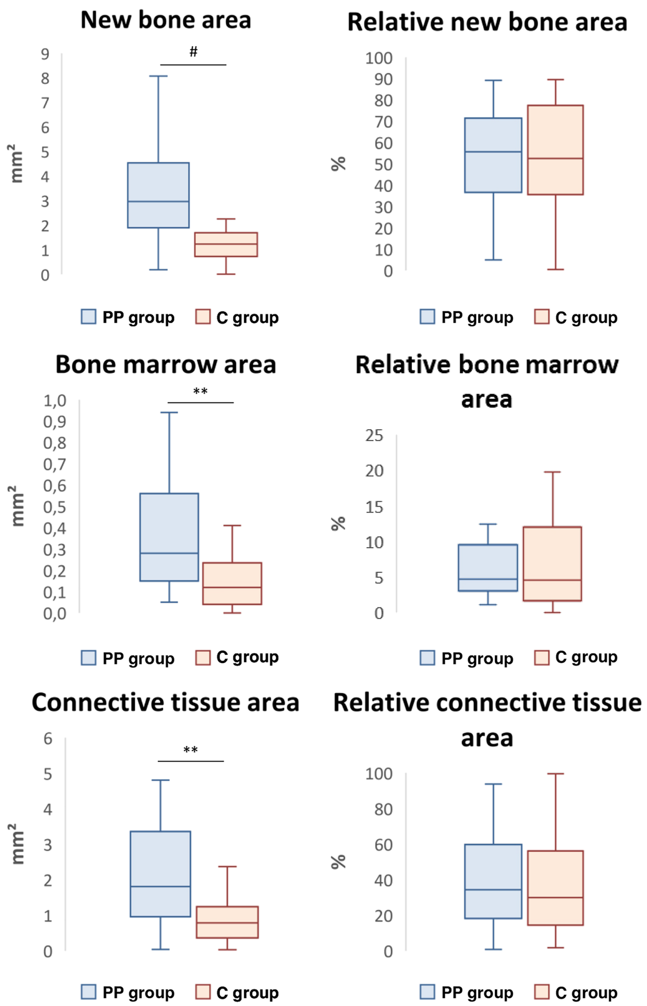

FIGURE 5 | Histomorphometric data of the area parameters analysed for newly formed bone (NB, mm^2), bone marrow (BM, mm^2) and connective tissue (CT, mm^2). The area volumetric density of new bone (%NB), bone marrow (%BM) and connective tissue (%CT) were measured. Statistical analysis was performed by the Mann–Whitney *U* test. Data are presented as box plots with median, means and interquartile ranges. $^{**}p < 0.01$, $^{\#}p < 0.001$.

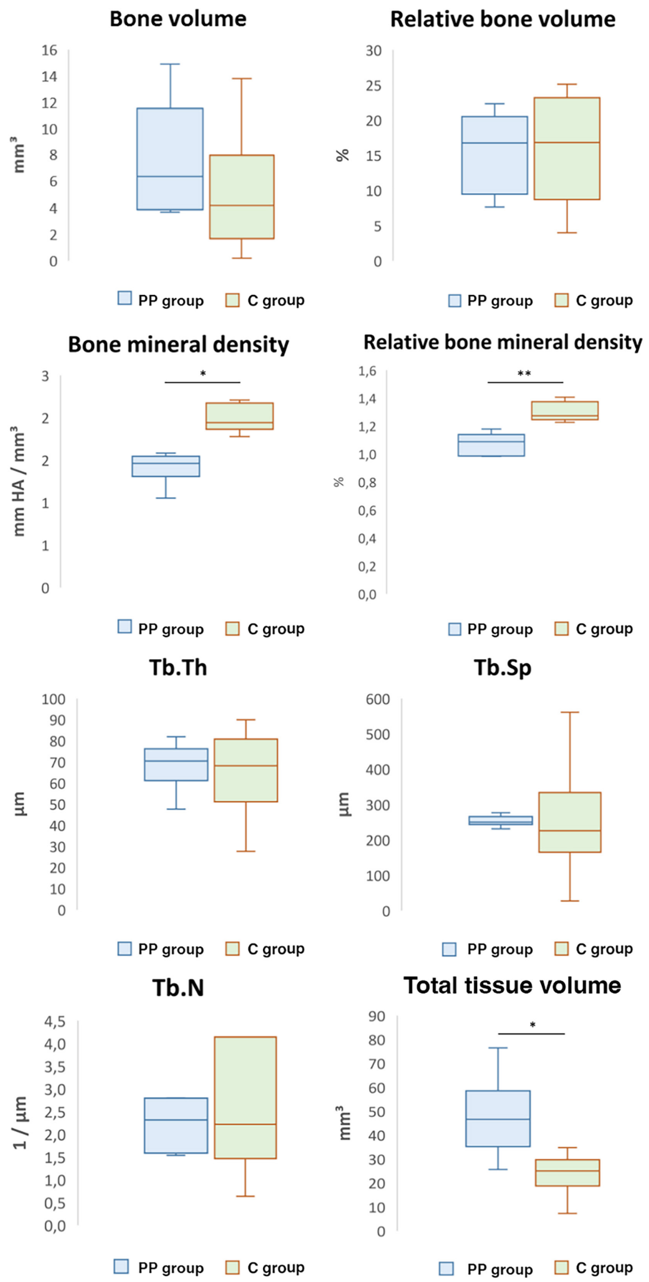

FIGURE 6 | Micro-CT analysis of the alveolar bone. Quantification of the parameters showing bone volume (BV, mm^3), ratio of the BV to the TTV (%BV), bone mineral density (BMD, mg HA/mm^3), ratio of the BMD to the density of the TTV (%BMD), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp) and total tissue volume (TTV, mm^3). Statistical analysis was performed by the Mann–Whitney *U* test. Data are presented as box plots with median, means and interquartile ranges. $^{*}p < 0.05$, $^{**}p < 0.01$.

the distraction plate was not included in the assessment of the present study.

The preservation of the periosteum during surgical procedures appears to be fundamental to maintain a periosteum-mediated bone regeneration and to optimize the treatment outcomes (Lin et al. 2014). To the best of our knowledge, periosteal distraction was applied for the first time for the vertical augmentation of the alveolar ridge in a pre-clinical study. The application of the distraction principle to the periosteum avoided the necessity of autogenous bone harvesting, but the presence of the distraction device in the mouth represented its major drawback. The external part could hinder mastication and facilitate accumulation of biofilm and food remnants. Nonetheless, devices like the one used in the present study have potential to stimulate bone formation from the pristine bone.

Mechanical stimulation of endogenous growth factors from the periosteum represents a simple and effective approach for enhancing bone regeneration (Correia et al. 2013). Maximizing the regenerative potential of endogenous tissues for therapeutics requires the development of novel biomedical approaches to recruit host cells and direct functional regeneration (X. Wang et al. 2018). The present study indicates the presumptive potential of an alternated protocol of periosteal distraction. Furthermore, the activation and relaxation of periosteum could activate distinct proliferation and differentiation pathways specific to the stress applied (Gabbay et al. 2006; L. Wang et al. 2020). The possible application of the present technique in a treatment of larger segmental bone defects may show some promise (Ransom et al. 2018). However, the design of the device would need further modifications to allow a submerged placement prior to clinical application. The role of morphogenes and transcription of osteogenic factors (Kusumbe, Ramasamy, and Adams 2014) remains to be determined in future studies.

Author Contributions

Nikola Saulacic: conception and design, data analysis and interpretation, drafting and critically revising the manuscript. **Niklaus P. Lang:** conception, interpretation of data and critically revising the manuscript. **Slavko Corluka:** design, data acquisition and analysis, manuscript drafting. **Maria Permuy Mendaña:** design, data acquisition and analysis, manuscript drafting. **Fernando M. Muñoz Guzón:** conception and design, interpretation of data, critically revising the manuscript. All authors gave their final approval and agree to be accountable for all aspects of the work.

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Ethics Statement

The current study was conducted in accordance with the European Communities Council Directive 2010/63/EU, approved by the Ethics Committee of the Rof Codina Foundation, Lugo, Spain (AELU001/66548/RX 1548438).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.