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Title: Comparative microcomputed tomography and histological analysis of the effects of a horizontal platelet-rich fibrin bone block on maxillary sinus augmentation. A preclinical in-vivo study.

Running title:

Effects of platelet-rich fibrin bone on sinus augmentation

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Key finding from the study

The novel horizontal platelet-rich fibrin (H-PRF) bone block can increase vertical bone gain and bone microstructure in the sinus augmentation process by inducing more angiogenesis, more osteoclasts, more new bone formation and less material residue, especially in the regions close to the previous bone plate.

Abstract

Background: While suggested to be effective in tissue regeneration, the effects of horizontal platelet-rich fibrin (H-PRF) bone block in sinus augmentation have not been verified in an animal model.

Methods: Twelve male New Zealand white rabbits that underwent sinus augmentation were divided into two groups: deproteinized bovine bone mineral (DBBM) only and H-PRF bone block. H-PRF was prepared at 700 × g for 8 min using a horizontal centrifuge. The H-PRF bone block was prepared by mixing 0.1 g DBBM with H-PRF fragments and then adding liquid H-PRF. Samples were collected after 4 and 8 weeks and analyzed using microcomputed tomography (micro-CT) for vertical bone gain of the sinus, bone volume/total volume (BV/TV) percentage, trabecular number (Tb.N), trabecular thickness (Tb.Th), and trabecular separation (Tb.Sp). Then, histological analyses were performed to investigate new blood vessels, material residue, bone formation and osteoclasts.

Results: Higher vertical bone gain of the sinus floor, BV/TV percentage, Tb.Th, and Tb.N and lower Tb.Sp were found in the H-PRF bone block group at both time points compared with the DBBM group. Higher amounts of new blood vessels and more osteoclasts were found in the H-PRF bone block group than in the DBBM group at both time points, especially in the regions close to the bone plate. More new bone formation and less material residue were observed in the H-PRF bone block group at 8 weeks.

Conclusions: H-PRF bone block showed greater potential for sinus augmentation by promoting angiogenesis, bone formation and bone remodeling in a rabbit model.

Key words: Platelet-rich fibrin, Bone formation, Sinus augmentation, Angiogenesis

Introduction

Dental implant placement in the posterior maxillae is usually difficult when there is vertical maxillary bone deficiency due to tooth loss and gradual sinus pneumatization.[1-3] To overcome these difficulties, sinus augmentation or short implants are often utilized.[4-6] The lateral window approach, proposed by Tatum[7] and described by Boyne and James[8] in 1980, is a predictable and frequently performed method to augment the maxillary sinus and allow implant placement in severely resorbed posterior maxilla regions. For both simultaneous implant placement and a two-stage approach, the survival rate of dental implants after sinus augmentation is higher than 95%, according to retrospective studies and systematic reviews.[9-12]

Bone graft materials commonly used in the bone augmentation process include allografts, autografts, xenografts or a combination with various growth factors.[13-15] Although it is considered the gold standard for bone augmentation, the high morbidity and unpredictable reabsorption rate encourage clinicians to use other bone substitutes.[16] Unlike other vertical bone defects, the dynamic air pressure exerted by breathing may stimulate the osteogenic potential of the Schneiderian membrane and bone resorption through an abnormal stimulus to grafted materials and bone.[17, 18] Therefore, the ideal bone substitute for sinus augmentation should have good volume maintenance and a suitable resorption rate. Demineralized bovine bone mineral (DBBM) has been widely used in clinical dental practice due to its osteoconductive properties and low resorption rate.[19] However, DBBM lacks osteogenic and osteoinductive properties, limiting its effects in maxillary sinus augmentation.[20] Horizontal platelet-rich fibrin (H-PRF) is a

novel PRF produced by horizontal centrifugation, resulting in abundant leukocytes and bioactive proteins entrapped in the fibrin network, exerting a slow but steady release of growth factors.[21] It has been reported that PRF could be used alone or in combination with graft materials in the treatment of socket preservation, periodontal defects, and sinus augmentation.[2, 22-26] By mixing liquid H-PRF and solid H-PRF with DBBM, the composite graft formed a moldable bone block with a short solidification time. This study hypothesizes that the novel H-PRF bone block not only has a certain mechanical strength but also displays high bioactivity, including angiogenesis and osteogenesis, due to the cellular active components in H-PRF,[27, 28] which is ideal for sinus augmentation. In vitro studies have proven that H-PRF can promote osteogenesis,[29,30] but its effect on bone regeneration in maxillary sinus augmentation has not been proven in vivo.

Therefore, the aim of the present study was to prepare and apply H-PRF bone block in a rabbit maxillary sinus augmentation model and to investigate the potential benefits of H-PRF bone block on bone formation through microcomputed examination and histomorphometric evaluation.

Materials and methods

Study design

Prior to the start of the experiments, rabbit management and experimental protocols were carried out in accordance with the ARRIVE guidelines for animal research and approved by the Ethics Committee at the School of Stomatology, Wuhan University,

People's Republic of China. The Ethics Committee for Animal Use approved protocol number B52/2020. Twelve male adult New Zealand rabbits (mean weight 2.5 to 3.0 kg, aged 6 months) were obtained and kept at a constant temperature to acclimate to the facility for approximately one week prior to surgery. The sample size was calculated using PASS 15.0 software (NCSS, USA). The analysis module Two-Sample T Test Assuming Equal Variance was used. The statistical design was based on comparing the bone formation area at 8 weeks after tricalcium phosphate and PRF+tricalcium phosphate were applied to the sinus elevation in rabbits. According to the results of the preliminary experiment, the mean rates of bone formation in the control groups and PRF groups were 29.9% and 41.5%, respectively. The standard deviation was set as 5%. Statistical significance was set as $\alpha = 0.05$, with a statistical power of 0.9 and a group allocation ratio of 1:1. With these parameters, the sample size needed for the current study was six in each group (n represents the number of sinuses). [31,32] All rabbits underwent bilateral sinus augmentation and were divided into two groups: the control group, which received DBBM (Geistlich Bio-Oss®, Geistlich Pharma North America, Inc., Wolhusen, Switzerland) only, and the treatment group, which received H-PRF bone block. Group assignment was performed using random number generation. The animals were allocated to the healing time point randomly. Considering that the rabbit model permits the use of both sides of the maxillary sinus, 12 rabbits allowed the collection of 24 samples, resulting in six data points for each group (n = 6). All rabbits were fed in separate cages and kept in a controlled environment at 22-26 °C with free access to food and water.

Surgical protocols

The rabbits were anesthetized with 20% urethane (Sinopharm Chemical Reagent Co., Ltd., Ningbo, China) at approximately 5 ml kg^{-1} body weight through central ear vein injection. After the skull was shaved and the skin was disinfected, a 1 cm mid-sagittal incision above the nose extending to eye level was made in the midline of the nasal bone, and a full-thickness flap, including the skin and periosteum, was elevated. The sinus lift procedure was performed according to previous studies.[29, 30]

Briefly, a standard-sized circular bone window was prepared bilaterally using a 6-mm diameter trephine (MICA kit™, Mega'gen Implant Co., Ltd., Seoul, Korea) under uninterrupted refrigeration with 0.9% sterile saline solution (Figure 1). Gentle drilling was conducted until the underlying Schneiderian membrane (SM) was visible through the thinned bone plate. To avoid membrane perforation, the SM was carefully elevated after the bone plate was removed. Each sinus was randomly assigned to the test group or the control group.

In the control group, approximately 0.1 g DBBM (particulate size, approximately 0.25 to 1.0 mm in diameter) was grafted. In the experimental group, two tubes of 6 ml whole blood were drawn from each rabbit. One tube was made of glass, and one tube was made of plastic; no anticoagulants or coagulants were added to any of the tubes. Then, the tubes were immediately centrifuged at 700 × g for 8 min using a horizontal centrifuge (Eppendorf® 022628012 (NA), Hamburg, Germany) based on previous studies to produce solid horizontal platelet-rich fibrin and liquid horizontal platelet-rich

fibrin separately. [21,31,32] The remaining H-PRF clots were cut into small pieces after carefully removing the red blood cell layer. A syringe was used to collect the yellow liquid H-PRF above the red blood cell layer. DBBM (0.1 g) was mixed with the solid H-PRF fragments and then mixed with 0.5 mL liquid PRF. After solidification, the bone block was placed in the prepared sinus openings, and then the bone plate was replaced to cover the sinus opening.

All metal devices, such as surgical scissors or tweezers, used in the surgery were sterilized. After the surgical procedures were completed, the skin and periosteum flap were sutured with 4-0 polyglactin 910 suture (Monocryl, AgnTho's, Lidingo, Sweden). All rabbits were medicated with 30,000 U/kg penicillin potassium (Huachu, Zhengzhou, China) for 3 days by intramuscular administration. The rabbits were euthanized using an overdose of anesthetics. The specific healing time was calculated by taking the day of the sinus augmentation operation as the first day. Six rabbits were randomly selected for sampling at 4 weeks, and the remaining rabbits were euthanized and sampled at 8 weeks.[34]

Radiographic assessment

All biopsy specimens were placed in a custom-made holder to ensure that the long axis of the drilled channel was perpendicular to the axis of the X-ray beam. The scanning parameters for micro-CT analysis were as follows (SkyScan 1176, Bruker, Kontich, Belgium): 55 kV/114 μA; integration time, 200 ms; 1024 reconstruction matrix; filter AI, 0.5 mm; and exposure time, 596 ms. Then, the cross-sectional

images were repositioned using the software DataViewer (Bruker, Kontich, Belgium) to evaluate the vertical increase in bone (mm) in the X-Y and X-Z axes. The morphometric parameters were calculated according to the methodology described by Fernanda and coworkers (2017). Based on the calculated histogram, the bone was defined to be in the 64–225 gray value range. Within the view of interest (VOI), the volume of the newly formed bone was calculated as a percentage. The sinus was identified based on the grayscale threshold setting. Radiographic measurements were made twice by a blinded, experienced examiner (S.Y.) using an automated digital method. Bone-related parameters, including the bone volume/total volume (BV/TV) percentage, trabecular number (Tb.N), trabecular thickness (Tb.Th), and trabecular separation (Tb.Sp), were analyzed using the CTAn program (Bruker, Kontich, Belgium) as previously described.[35]

Histological procedures and histomorphometric analyses

After micro-CT scanning, all specimens were decalcified using 10% ethylenediaminetetraacetic acid solution for 1 month. Then, the samples were rinsed with distilled water, dehydrated with an ethanol gradient (25%, 50%, 75%, 100%) for 2 hours at each concentration and subsequently embedded in paraffin as described previously.[36] A vertical incision was made along the diameter of the circular bone window area previously prepared by the trephine. Serial sections 5 μm thick were cut using a semiautomatic vibrating blade microtome (Leica Biosystems, Wetzlar, Deutschland) in the coronal plane. The central-most 15 serial sections of the sinus were

harvested and mounted on polyline-coated microscope slides. There were six sinuses in each group for radiographic assessment at each time point (n=6). Each maxillary sinus specimen was scanned and then embedded in paraffin, sliced and stained for histological experiments. The regions of interest (ROIs; size: 1 mm × 1 mm) were defined at three different regions: (i) close to the antrostomy (bone region), (ii) in the center of the grafts (middle region), and (iii) subjacent to the sinus mucosa (submucosa region). The imaging and analysis were applied under 20× magnification in all regions separately. [37] The formation of new bone, residual DBBM material and number of vessels in the sinus areas were monitored using hematoxylin and eosin (H&E) staining and Masson's trichrome (MT) staining. The existence and number of osteoclasts during the 4-week and 8-week healing periods were investigated using TRAP staining (TRAP staining kit, Solarbio, Beijing, China) according to the manufacturer's protocol. The number of vessels and osteoclasts was counted on ten consecutive sections of each specimen. The following histomorphometric analyses were recorded: (i) the area of total augmentation (TA; mm^2), (ii) the percentage of NB within TA (NBA%), and (iii) the percentage of RM within TA (RMA%). In each section, six representative fields (1024 x 1536 pixels) were studied and averaged under 20× magnification. The above histomorphometric analyses were conducted by an experienced blinded examiner (S.Y.).

Statistical assessment

The statistical analysis was performed using GraphPad Prism software 8.0.2. The data are expressed as the mean ± standard deviation (SD) for the bone volume/total volume

(BV/TV) percentage, trabecular number (Tb.N), trabecular thickness (Tb.Th), and trabecular separation (Tb.Sp) and were analyzed using two-way ANOVA. The analyses of the vertical increase in bone in the X-Y and X-Z axes and the number of new blood vessels and osteoclasts were also performed using two-way ANOVA. *P < 0.05, **P<0.01, and ***P<0.001 were considered statistically significant.

Results

General findings

Only slight soft tissue edema was observed in a few rabbits. During the sinus augmentation surgery and the healing period, no adverse events or complications, such as Schneiderian membrane perforation and wound dehiscence, were noted in either group. The experimental model of the maxillary sinus in rabbits and the surgical procedure are shown in Figure 1.

Radiographic analysis

Vertical bone gain

Micro-CT views of the augmented sinus and statistical analysis of the quantitative parameters are shown in Figure 2 A-C. The elevated height was defined as the maximal distance between the sinus bony floors and raised nasal dorsum in the augmented space. To gain the ideal effect of vertical bone augmentation during the sinus lift process, bone substitutes should be stable enough to resist the mucosal pressure caused by respiratory movement. Adequate elevation height and bone integration were the main assessments

in clinical treatment. Therefore, by controlling the consistent quality of DBBM used in the H-PRF bone block group and the DBBM group, the change in vertical bone gain can better reflect the effect of H-PRF in maxillary sinus elevation. Both the H-PRF bone block group and the DBBM group showed some degree of absorption at 8 weeks compared with 4 weeks. The vertical bone gain was 4.642 ± 0.287 mm in the H-PRF bone block group and 4.034 ± 0.694 mm in the DBBM group at 4 weeks. The vertical bone gain was 4.148± 0.237 mm in the H-PRF bone block group and 3.893±0.238 mm in the DBBM group at 8 weeks. The vertical bone gain of the H-PRF bone block group was slightly higher than that of the DBBM group in both the X-Y and X-Z axes ($p <$ 0.01) (Figure 2 D-E).

Quantitative analysis related to bone regeneration

The results of the quantitative analysis are shown in Figure 2 F-I. After 4 weeks of healing, BV/TV (%) was 54.610±8.138 in the DBBM group and 65.225±8.623 in the H-PRF bone block group (p=0.018). After 8 weeks, BV/TV (%) was 70.571±4.251 in the DBBM group and 82.423±2.124 (p=0.009) in the H-PRF bone block group. The trabecular thickness (Tb.Th, mm) increased from 0.249±0.027 at 4 weeks to 0.300±0.050 at 8 weeks in the DBBM group and from 0.278±0.031 at 4 weeks to 0.362±0.016 at 8 weeks in the H-PRF bone block group. The difference between the two groups was statistically significant at 4 weeks (p= 0.033) and 8 weeks (p<0.001). The trabecular number (Tb.N, mm^{-1}) was 1.944±0.189 and 2.197±0.122 in the DBBM group and 2.333±0.192 and 2.523±0.072 in the H-PRF bone block group at 4 and 8

weeks, respectively (p<0.001 and p= 0.03, respectively). The trabecular separation (Tb.Sp, mm) was 0.217±0.031 and 0.204±0.028 in the DBBM group and 0.171±0.013 and 0.149±0.014 in the H-PRF bone block group after 4 and 8 weeks of healing, respectively (p=0.005 and p=0.001, respectively).

Histomorphometric analysis

Angiogenesis

Neoangiogenesis in the sinus was investigated by H&E staining (Figure 3). The number of new blood vessels was counted in 6 fields of each group in three different areas. Near the bony floor, the average number of new vessels in the H-PRF bone block group at 4 and 8 weeks was 27.167±8.208 and 29.000±3.578, respectively, and that in the DBBM group was 16.167±5.636 and 22.500±6.834, respectively. The average number of new vessels in the center of the elevated space in the H-PRF bone block group at 4 and 8 weeks was 17.667±7.118 and 21.500±5.468, respectively, and in the DBBM group, it was 12.833±0.983 and 20.333±3.266, respectively. Subjacent to the sinus mucosa, the average number of new vessels in the H-PRF bone block group at 4 and 8 weeks was 20.000±3.847 and 22.167±3.764, respectively, and 12.000±1.414 and 21.833±4.070 in the DBBM group, respectively (20×).

It can be concluded that angiogenesis after surgery is most active in the area near the bone plate, and more new blood vessels form as the healing time increases from 4 weeks to 8 weeks. In general, the H-PRF bone block group showed a higher number of new blood vessels than the DBBM group.

New bone formation

The DBBM graft was pink in H&E staining and red in MT staining, differentiating it from the surrounding tissue (Figures 3 and 4). Mature bone was stained red, and newly formed bone was stained blue in MT staining (Figures 3 and 4). No signs of inflammation were shown by H&E and MT staining under light microscopy in either the DBBM group or H-PRF bone block group. All groups showed the smallest new bone area (NBA) formation at the center of the bone graft, while more NBA near the bone walls and Schneiderian membrane was observed at 4 weeks and 8 weeks (Figure 4 M-O). Between the two groups, a larger NBA% and smaller RMA% were observed in the H-PRF bone block group at 8 weeks (Figure 4).

Evaluation of residual material and bone remodeling

At 4 weeks and 8 weeks, NBA% was higher in the H-PRF bone block group than in the DBBM group, while RMA% was lower ($p<0.05$ at 4 weeks) (Figure 4). The tartrate-resistant acid phosphatase (TRAP)-stained osteoclasts (arrows) are shown in Figure 5. The total number of TRAP-stained osteoclasts at 4 and 8 weeks was 45.667±10.520 and 36.333±4.803, respectively, in the experimental group and 33.833±5.707 and 39.000±7.668, respectively, in the DBBM group (Figure 5). The total number of osteoclasts decreased from 4 to 8 weeks in both the DBBM and H-PRF bone block groups. The most significant difference between the two groups was found mainly near the bony floor of the sinus (Figure 5).

Discussion

Maxillary sinus augmentation has been widely used to gain bone height for dental implant placement with a low complication rate and higher implant survival rate.[38] The elevated space demands sufficient bone graft material filling to obtain adequate bone support. As the maxillary sinus of the rabbit has a well-defined ostium similar to that of humans, a rabbit sinus augmentation model was used in this study.[39] When the Schneiderian membrane is elevated, an irregular cavity forms, and the air pressure affects the augmented volume. It is important for bone substitutes to fill the irregular bony cavity and resist pressure to maintain long-term vertical stability[40]. Therefore, a grafting material with a proper resorption rate may maintain the graft height and has advantages in sinus augmentation. However, it is difficult for loose granular graft materials to remain stable under pressure from the mucosa, resulting in material collapse or requiring a large amount of bone substitute utilization for maxillary sinus augmentation.

Platelet-rich fibrin (PRF) has beneficial effects on tissue regeneration healing by providing three-dimensional scaffolds, live cells, and a source of growth factors such as platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-β), insulin-like growth factor (IGF) and vascular endothelial growth factor (VEGF).[41] Horizontal platelet-rich fibrin (H-PRF), prepared by horizontal centrifugation, is considered to have more uniform cell separation than traditional platelet-rich fibrin prepared by fixed-angle centrifugation.[33] By adding liquid H-PRF to the mixture of

solid H-PRF fragments and DBBM, a moldable sticky bone block forms after solidification. The present study showed that the H-PRF bone block has certain elastic and compressive properties, with a higher ability to promote osteogenesis in osteoblasts in vitro, but its effect on bone regeneration after maxillary sinus elevation has not been proven in vivo.[42]

Therefore, in this study, we evaluated the potential effects of H-PRF on bone formation after maxillary sinus augmentation in a rabbit model by means of microcomputed examination and histomorphometric evaluation. As osteogenic factors mainly arrive from the native bone at the base of the sinus and Schneiderian membrane,[42,43] ossification in the center area is usually more difficult. Adding horizontal platelet-rich fibrin might be beneficial for gradually releasing cytokines.

The results of microcomputed tomography (micro-CT) showed that the vertical height was slightly increased in the H-PRF bone block group compared with the DBBM group, although bone resorption was observed in both groups at 8 weeks compared with 4 weeks. This might be the result of the space maintenance ability of the H-PRF bone block compared with granular DBBM provided by the natural fibrin bioscaffold in H-PRF.

BV/TV indicates the ratio of bone tissue volume to tissue volume, which can directly reflect changes in bone mass. It was observed that the addition of H-PRF increased bone mass at both 4 and 8 weeks. Trabecular thickness (Tb.Th, mm), trabecular number (Tb.N, mm^{-1}), and trabecular separation (Tb.Sp, mm) within the augmented sinus are three important analysis indicators. The H-PRF bone block group generated more

trabecular bone, the thickness of trabecular bone was larger, and the trabecular bone was denser. This phenomenon can be explained by the release of growth factors from within the H-PRF.[45, 46]

By hematoxylin and eosin (H&E) staining and Masson's trichrome (MT) staining, the amount of residual material, new bone and bone maturation, and the number of blood vessels could be visually observed. The activation and complex interaction between angiogenic and osteogenic pathways are crucial during bone repair and bone remodeling processes. Several studies have demonstrated that angiogenesis precedes the onset of osteogenesis. Therefore, we analyzed and compared the neovascularization of each group, and the results showed that the difference in neovascularization between the two groups was more pronounced at 4 weeks, regardless of observation location. At the same time, we observed more new bone and mature bone formation near the bone plate and mucosa and more DBBM residue in the center region, which were consistent with the results of previous studies.

Previous histological studies on bone regeneration have shown significant blood clots and inflammatory responses in the bone augmentation area during the first two weeks. [47] Subsequently, fibrous granulation tissue dominated the graft bed, with reduced inflammatory cells and increased osteoclast activity. During the revascularization process, the original mesenchymal cells gradually differentiate into osteoblasts. [48] At the same time, the bone graft is progressively absorbed by osteoclasts in a circular process. Bone remodeling is a synchronized complex process of bone matrix formation by osteoblasts and absorption by bone osteoclasts.[48] Tartrate-resistant isoenzymes are

present in bone-resorbing cells and osteoclasts and have been isolated and characterized biochemically.[49] In our study, many TRAP-stained osteoclasts were observed on the surface of the elevated sinus membrane, the floor of the sinus cavity, and the floor of the repositioned bony window. According to this study, H-PRF was beneficial for the formation of vessels and the number of osteoclasts and showed better spatial maintenance to resist respiratory pressure, thereby accelerating the bone regeneration process in sinus augmentation. At the same time, these effects were more significant in the area near the bone plate and Schneiderian membrane.

By adding solid H-PRF fragments and liquid H-PRF, granular DBBM can be transformed into a bone block with plasticity. Compared with the control group, the experimental group obtained better bone formation even when the same quality of DBBM was used, which is conducive to saving patients the cost of bone substitutes clinically.

Limitations of the study:

It is known that healing in phylogenetically lower animals is faster than in humans. Clinical studies are also needed to further verify the benefits of H-PRF bone block in maxillary sinus lift practice.

Conclusion

The comparative micro-CT and histomorphometric results showed that the novel H-PRF bone block promoted the formation of blood vessels, bone remodeling process and

new bone regeneration compared with DBBM only in sinus augmentation, providing evidence for the clinical utilization of H-PRF bone block to improve maxillary sinus augmentation effects.

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Author contribution

S.Y. and Y.T. contributed equally. S.Y., Y.W., Y.Z. and Z.Y. conceived and designed the research. S.Y., Y.W., Y.T., Y.W., M.F., S.L., G.T. and Z.Y. performed the experiments. S.Y., Y.W. and Y.W. performed the analysis. S.Y. and Y.W. interpreted the data and wrote the manuscript. S.Y., Y.W., R.J.M., Y.Z. and Z.Y. critically revised the manuscript. All authors approved and agreed to the final revision.

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The data that support the findings of this study are openly available.

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The authors stated that there is no conflict of interest that should be disclosed in this

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All participants provided informed consent prior to treatment in the study.

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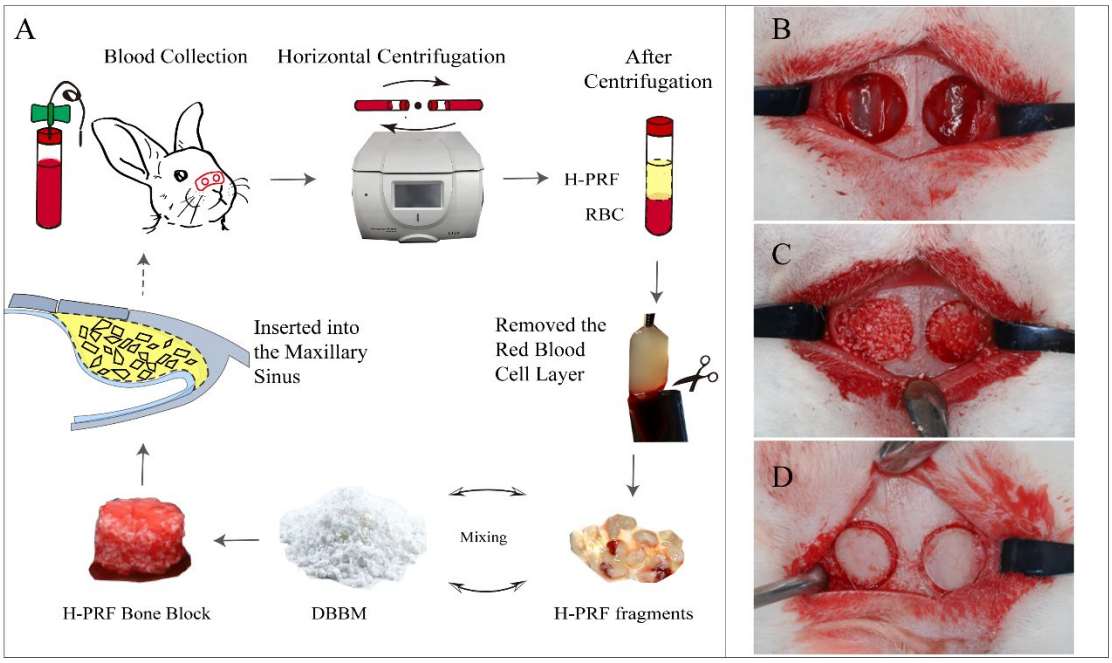

Figure. 1 The preparation process of the rabbit sinus augmentation model (a) Schematic representation illustrating of the rabbit maxillary sinus augmentation model using horizontal platelet-rich fibrin (H-PRF) and DBBM. (b) The flaps were elevated to expose the nasal bone and the partial osteotomies were removed. (c) Left: sinus cavity filled with DBBM; Right: sinus cavity filled with DBBM and H-PRF. (d) Covered with original osteotomies.

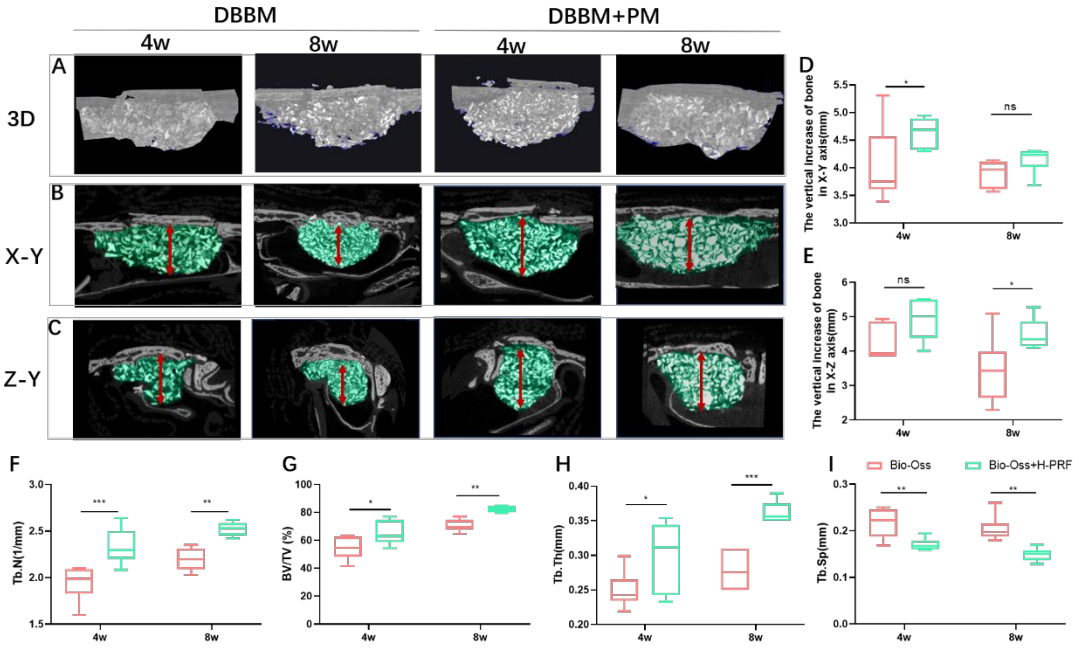

Figure. 2 Micro-computed tomography (CT) views of augmented sinuses and statistical analysis of quantitative parameters. (A) 3D image of augmented sinuses.(B-C) The X-Y and Z-Y axis view of augmented sinuses separately. The red arrows show the measured distance. (D-E) the vertical increase of bone in X-Y and X-Z axis separately (F) the volume of bone per unit volume of the total augmented volume (BV/TV, %), (G) trabecular thickness (Tb. Th, mm), (H) trabecular number (Tb. N, mm^{-1}), and (I) trabecular separation (Tb. Sp, mm) within the augmented sinus (n=6). *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$. (n=6).

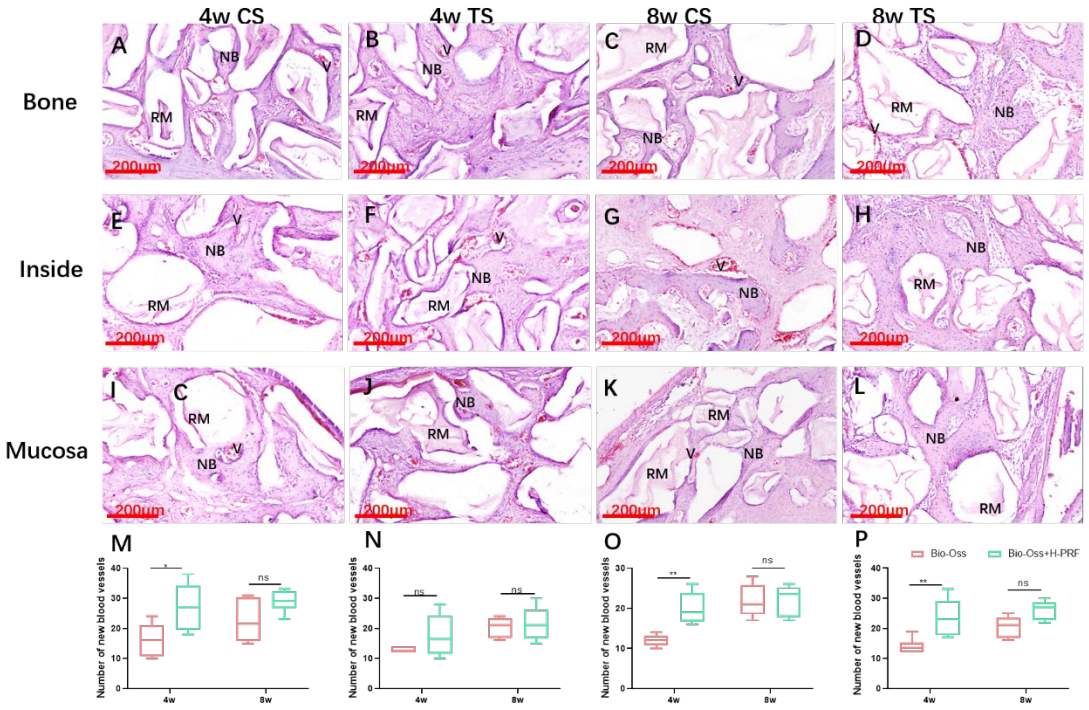

Figure. 3 Photomicrograph of hematoxylin and eosin (H&E) staining showing the sinus augmentation region at 4 weeks (A, B, E, F, I, J) and 8 weeks (C, D, G, H, K, L). Three different areas were chosen in each groups for comparison: Bone (close to the antrostomy); Inside (in the center of the elevated space); Mucosa (subjacent the sinus mucosa). B, DBBM residuum; NB, newly formed bone; rB, replaceable bone; V, vessel. (×20); Neovascularization of different areas (M) close to the antrostomy; (N) in the center of the elevated space; (O) subjacent the sinus mucosa. (P) the total situation calculated by hematoxylin and eosin (H&E) staining at week 4 and week 8 (n=6). ns: not statistically significant versus control group, *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

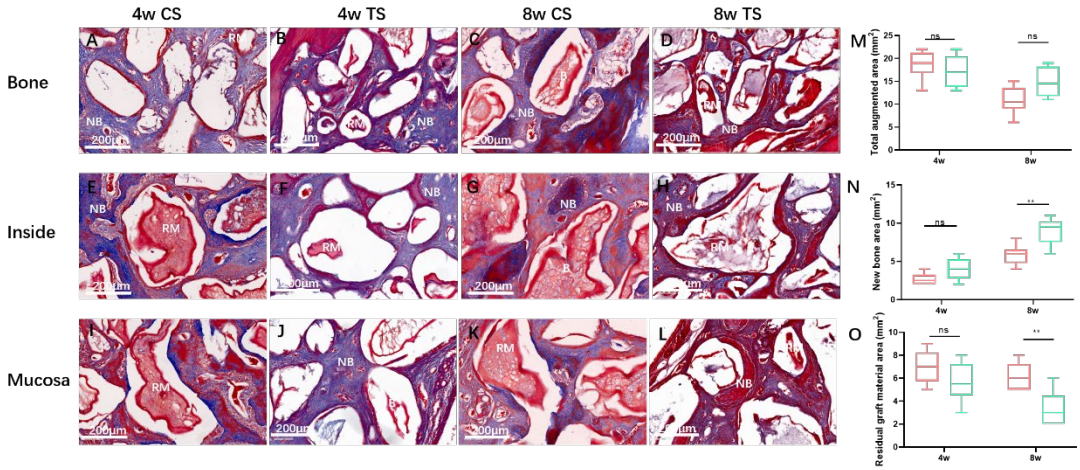

Figure. 4 Photomicrograph of Masson's trichrome (MT) staining showing the sinus augmentation region at 4 weeks (A, B, E, F, I, J) and 8 weeks (C, D, G, H, K, L). Three different areas were chosen in each group for comparison: Bone (close to the antrostomy); Inside (in the center of the elevated space); Mucosa (subjacent the sinus mucosa). The mature bone was defined as a red color structure containing osteocytes in MT stain B, DBBM residuum; NB, newly formed bone; rB, replaceable bone; V, vessel (×20). Histomorphometric analysis within the total augmented area (mm2). (M)Total augmented area (TAA, mm2) within the region of interest (ROI), enclosed by the antral bone wall, Schneiderian membrane and the window.

(N) Newly formed bone area (NBA, mm2) within the TAA. (O)Residual graft material area (RMA, mm2) within the TAA. ns: not statistically significant versus control group, *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

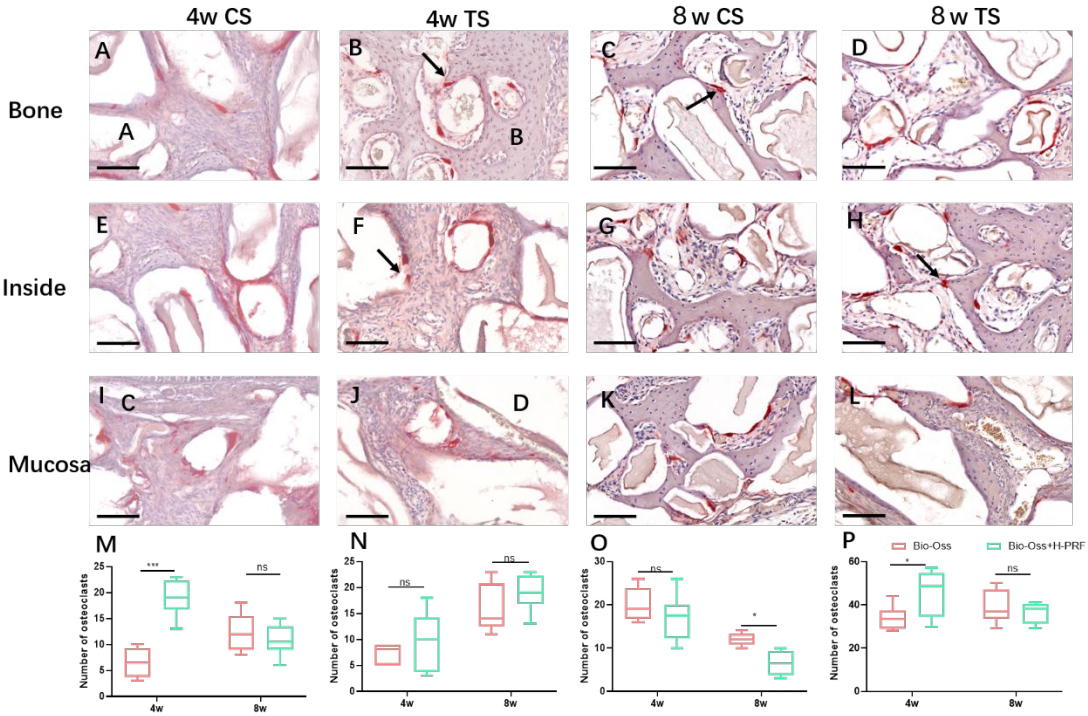

Figure.5 Photomicrograph shows tartrate-resistant acid phosphatase (TRAP)-stained osteoclasts (arrows) in the sinus at 4 weeks and 8 weeks in (A-D)bone regions, (E-H) inside region, (I-L) near mucosa region (×20). Histomorphometric measurement of the number of osteoclasts at 4 and 8 weeks in different areas: (M) close to the antrostomy; (N) in the center of the elevated space; (O) subjacent the sinus mucosa. (P) the total calculation(n=6). ns: not statistically significant versus control group, *$p < 0.05$ and ***$p < 0.001$.