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Hyperbaric Oxygen Combined with PRF on the Repair Effects of Calcined Bovine Bone to Periodontal Bone Defects and the Expression of RANKL/OPG

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Abstract

Objective: To investigate the effect and mechanism of Hyperbaric Oxygen Therapy (HBO) combined with Platelet-Rich Fibrin (PRF) on the treatment of periodontal bone defects using calcined bone (CBB).

Methods: A total of 150 patients with chronic periodontitis and bone defects were selected and divided into three groups after calcined bone grafting: the HBO-PRF group, the PRF group, and the control group, with 50 cases in each group. The Gingival Index (GI), Periodontal Pocket Depth (PD), and Clinical Attachment Level (AL) were recorded before surgery and at 6 and 12 months post operation. CBCT and periapical x ray were taken to measure bone density and bone filling, and the rate of bone improvement was calculated. Enzyme-Linked Immunosorbent Assay (ELISA) was used to determine the expression of Osteoprotegerin (OPG) and Receptor Activator of Nuclear Factor-kappa B Ligand (RANKL) in Gingival Crevicular Fluid (GCF) before surgery and at 6 and 12 months post operation, and the Keratinized Gingival Width (KGW) was measured at different time points.

Results: The HBO-PRF group showed significantly reduced of the GI, PD, and AL at 6 and 12 months post operation compared to the PRF group and the control group. The reduction in KGW was significantly lower than that of the PRF group at 1, 3, 6, and 12 months post operation. The HBO-PRF group had a significantly higher bone density and bone fill improvement at 6 and 12 months compared to the PRF group and the control group. The GCF OPG was significantly higher than that of other groups, while RANKL and the RANKL/OPG were significantly lower.

Conclusion: The combination of HBO and PRF can significantly improve the therapeutic effect of calcined bone in repairing periodontal bone defects, and promote periodontal bone regeneration. And increase the KGW and the gingival papillae height, and improve black triangles. The mechanism is related to its regulation of the RANKL/OPG signaling pathway.

Keywords: Hyperbaric oxygen; Platelet-rich fibrin; Periodontal bone defect/regeneration; Keratinized gingival width; RANKL/oPG; Gingival papilla height.

Introduction

Periodontal bone defects are the most common problems in oral diseases and are the main cause of tooth loss, which seriously affects patients' chewing function and aesthetics. Traditional treatment methods such as flap curettage and bone grafting are effective but have certain limitations, such as poor bone regeneration ability, long healing time, and many complications. In recent years, Platelet-Rich Fibrin (PRF), as an autologous platelet concentrate, has attracted widespread clinical attention due to its high concentration of platelets, growth factors, and fibrinogen, which significantly promote the regeneration and repair of bone and soft tissues. The preparation method of PRF is simple and easy to operate, and it has affinity, no immune rejection, and therefore has received extensive attention in the clinic [1,2]. Clinical studies have shown that the combination of PRF with artificial bone powder treatment can improve the total effective rate and the height of bone formation [3].

Systematic reviews by Miron RJ have found that PRF has advantages in promoting bone regeneration and reducing complications in the repair of periodontal bone defects [4,5]. In addition, Hyperbaric Oxygen Therapy (HBO) can enhance cell metabolism and repair ability by increasing tissue oxygen concentration, playing an important role in promoting wound healing and bone regeneration [6].

However, there are few reports on the efficacy and mechanism of HBO combined with PRF in the repair of periodontal bone defects. Therefore, the application of HBO in combination with PRF may provide a new and effective treatment strategy for the repair of periodontal bone defects. Based on this, further research on the role of HBO combined with PRF in the repair of periodontal bone defects and its relationship with the expression of RANKL and OPG has important clinical significance and theoretical value.

Materials and methods

Patient selection: 159 patients with severe chronic periodontitis who met the inclusion criteria for this experiment were selected from those visiting the Stomatology Center of Changhai Hospital, Naval Medical University (one tooth was chosen per patient as the subject of the study, meeting the conditions for bone grafting). There were 79 male and 80 female patients, aged 38 to 61 years (average 48.5 years old). All patients underwent debridement and implantation of Calcined Bovine Bone (CBB) on the basis of periodontal basic treatment. According to the experimental design, patients were randomly divided into three groups: the HBO-PRF group, the PRF group, and the control group, with 53 cases in each group (Figure 1). This study was approved by the Ethics Committee of Changhai Hospital, Naval Medical University, and the patients signed informed consent forms.

Inclusion criteria: At the one-month follow-up after periodontal basic treatment, there should be at least one quadrant in the oral cavity with a periodontal pocket depth (PD)≥6 mm and a loss of periodontal attachment (AL)≥3 mm, and X-ray examination should show a clear infrabony pocket (grade 2 or higher bone absorption). Platelet count is greater than 100×10^9/L.

Exclusion criteria: Patients with severe systemic diseases, women in pregnancy or lactation, those who have taken medication affecting platelet function within 3 months before surgery, and patients with smoking and alcohol abuse.

Preparation of PRF: At 0.5 hours before surgery, 5 mL of venous whole blood was quickly drawn using a sterile vacuum glass tube (without anticoagulant), and immediately placed in a TLXJ-IIC centrifuge (An Ting Scientific Instrument Factory, Shanghai, China). It was then centrifuged at 3000 r/min for 10 minutes (with a centrifugal radius of 10 cm), and left to stand for 3 to 5 minutes. At this time, the centrifuge tube is divided into three layers: the uppermost layer is the supernatant, the lowest layer is the red blood cell layer, and the middle yellowish gel layer is the PRF. After centrifugation, the centrifuge tube is placed in a 37°C water bath for later use.

Treatment plan: Under local anesthesia, a full-thickness flap procedure was performed, the flap was raised to expose the bone defect area, and after debridement and root surface planing, the root surface was treated with minocycline for 3 minutes, followed by rinsing with saline. According to the experimental design, patients were divided into three groups (Figure 1). (1) HBO-PRF group: PRF was pressed into a membrane using sterile gauze, evenly divided into two parts, one part was prepared into fragments and mixed with the bone graft particles of CBB (Shaanxi, Ruisheng Biotechnology Co., Ltd., production batch number: 161202) and filled into the bone defect, the other PRF membrane was placed on the surface of the transplanted bone powder, the flap was repositioned and sutured, and the wound was dressed, starting from the second day after surgery, the patient received 0.25 MPa HBO exposure for 1 hour a day, five times a week, for a total of two weeks. (2) PRF group: CBB particles were implanted into the bone defect area, covered with a PRF membrane, and the wound was sutured. (3) Control group: After implanting CBB particles, the wound was sutured. Sutures were removed two weeks after surgery, and no periodontal probing was performed within six months after surgery. The patient took oral cefaclor capsules 0.5 g and metronidazole tablets 0.4 g, three times a day for one week, used Xipayi Guqin liquid (Xinqikang Pharmaceutical Co., Ltd.) for mouth rinsing, 3ml each time, three times a day, for six weeks, and avoided biting hard objects in the surgical area within four weeks.

Clinical indicator measurements: Periodontal clinical parameters were assessed at baseline, and at 6 and 12 months post-operation by two calibrated clinicians with a high level of agreement (κ>0.80) when collecting periodontal parameters. The following parameters were measured:

(1) The Gingival Index (GI) [7], which assesses the inflammation of the gingiva. (2). Probing Depth (PD), measured from the free gingival margin to the bottom of the pocket using a periodontal probe (Hu-Friedy Manufacturing, Chicago, IL, USA). (3). Attachment Loss (AL), measured from the enamel-cemental junction to the bottom of the pocket using a periodontal probe. GI, PD and AL were recorded at six sites per tooth (mesio-buccal, buccal, disto-buccal, disto-lingual, lingual, and mesio-lingual) around the teeth under investigation [8].

Measurement of keratinized gingival width: The KGW at the central buccal aspect of the experimental teeth in the surgical area was measured using a periodontal calibrated probe before surgery, and at 2 weeks, and 1, 3, 6, and 12 months post operation. Each site was measured twice, and the average value was taken. The change of KGW= (the preoperative KGW) - (the postoperative KGW).

Bone density assessment: Cone Beam Computed Tomography (CBCT, i-CAT 17- 19, Kavo, USA) was performed before surgery and at 6 and 12 months post operation. Bone density (BD) in the grafted area was measured using the iCATVision software (Version 1.9.3.14, USA) and expressed in Hounsfield units (HU).

Bone height measurement and comparison of bone improvement degree: X-ray films of the experimental teeth were taken, and the X-ray images of the experimental teeth were imported into a computer, then transferred to the Digimizer version 4.2 software (MedCalc Software, Belgium). Set the length of the tooth in the image to 10 (unit), and measure the alveolar bone height before surgery and at 6 and 12 months post- surgery using the software. The improvement rate of the alveolar bone height of the experimental teeth at 12 months was calculated according to the following formula, and the results were categorized as: (1) mild improvement, where the change of alveolar bone height is less than 50%; (2) moderate improvement, where the alveolar bone height increases by more than 50% but less than 150%; (3) significant improvement: where the alveolar bone height increases by more than 150%. The alveolar bone height expressed in units (u).

The formula is as follows:

Alveolar Bone Height Improvement Rate= (Bone Height at 12 Months Post- therapy-Pre-thrapy Bone Height) ÷ (Pre-thrapy Bone Height) ×100%.

Significant Improvement Rate= (Number of Moderate Improvement Cases + Numb er of Significant Improvement Cases) ÷ (Total Number of Cases) ×100%.

Measurement of gingival papilla height and black triangle area: Digital photographs of the gingival papilla recession sites of the experimental teeth were taken before treatment and at 12 months post-treatment using a digital single-lens reflex camera. The camera lens was positioned perpendicular to the labial surface of the long axis of the target tooth for the photograph. The photographs were then imported into the Digimizer version 4.2 software (MedCalc Software, Belgium). The software's line length calibration function was used to measure distances and areas based on the clinical photographs. The baseline was drawn from the Cervical Enamel-Dentin Junction (CEJ) of the adjacent teeth on the mesial and distal sides of the target gingival papilla (or the gingival margin instead) to the highest point on the root side, and a perpendicular line was drawn from the highest point of the gingival papilla to the baseline, indicating the Gingival Papilla Height (GPH) value. The image of the scale ruler in the photograph was used to calibrate the numerical value (principle: by clicking the calibration button and selecting two points on the ruler with a 10 mm interval, set the distance as 10 mm in the pop-up box. Then click on any two endpoints of a line segment, and the software's data measurement list will display the corresponding length value for this distance). The black triangle area (BTA) was outlined, and the area was automatically calculated by the software (Figure 2). Two examiners separately calculated the actual GPH and the BTA, and the average values were taken [9].

Detection of RANKL and OPG in GCF: GCF was collected before surgery and at 6 and 12 months post operation. A cotton roll was used to isolate moisture, and the area was gently blown for 10 seconds. A pre-weighed filter paper strip (10 mm x 2 mm) was gently inserted into the periodontal pocket from the site with the deepest probing depth until slight resistance was felt, left in place for 30 seconds, and then removed. After 10 seconds, the sampling was repeated; if the filter paper had bloodstains, the sampling was discarded and redone. A total of three samples were taken to form one specimen. After removal, the specimen was weighed and converted into volume (μL) according to the specific gravity (1 mg/μL). The weighed samples were immediately placed into an EP tube containing 500 μL of PBS buffer solution and stored at -80°C.

The samples were taken out from -80°C and naturally thawed at room temperature, then sonicated at 0°C for 1 hour (800 W, with a working time of 20 seconds and an interval time of 10 seconds). After that, the samples were centrifuged at 4°C at 5000 r/min for 5 minutes, the filter paper was removed, and

the supernatant was taken. The RANKL and OPG content in the supernatant was measured strictly according to the instructions of the ELISA kit (Shanghai Jidun Biological Co., Ltd.). Standard wells, blank wells, and sample wells were set on the enzyme-labeled plate, and the absorbance (OD) values were measured at a wavelength of 450 nm to calculate the sample concentration.

Statistical analysis: The data were statistically analyzed using IBM SPSS 23.0 (IBM Corp., Armonk, NY, USA). The outcome assessment and data analysis were performed by individuals blinded to group assignment information. The outcome assessment and data analysis were carried out by an author blinded to the group allocation information. Results were presented in the form of mean ± standard deviations. We used one-way ANOVA in combination with Paired t-test, independent samples t-test, Chi-Square Test and rank sum test (Wilcoxon) to compare the difference of multiple groups. Statistical significance was set at $p<0.05$.

Results

A total of 159 patients in this group completed the clinical surgery. Among them, in the HBO-PRF group of 53 cases, 1 case did not complete HBO treatment, and 2 cases were lost to follow-up. In the PRF group of 53 cases, 3 cases were lost to follow-up, and in the Control group of 53 cases, 1 case had postoperative infection, which improved after treatment with metronidazole tablets and cefaclor capsules and was subsequently withdrawn from the experiment, and 2 cases were lost to follow-up. 150 cases in this study completed the clinical research and data analysis.

Comparison of periodontal clinical indices: In the HBO-PRF group, GI, PD, and AL were significantly reduced compared to the PRF group at 6 and 12 months postoperatively. The PRF group showed significant differences in GI, PD, and AL compared to the Control group at 6 and 12 months postoperatively (Table 1).

Comparison of keratinized gingival width: The changes in KGW in the HBO- PRF group and the PRF group were significantly less than those in the Control group at 1, 3, 6, and 12 months postoperatively. There were significant differences in the changes of KGW at 3, 6, and 12 months compared to 1 month postoperatively, and no significant differences in the changes of KGW at 6 and 12 months compared to 3 months postoperatively. The changes in KGW in the HBO-PRF group were significantly less than those in the PRF group at 1, 3, 6, and 12 months (Table 2).

Bone density comparison: At 6 and 12 months post operation, there were significant differences in bone density (BD) among the three groups compared to preoperative levels. The BD in the HBO-PRF group was significantly higher compared to the PRF group and the Control group at 6 and 12 months post operation. The HBO-PRF group showed a marked increase in BD at 6 and 12 months compared to the PRF group (Table 3).

Comparison of bone fill improvement efficacy: The HBO-PRF group showed a significantly greater bone fill height at 6 and 12 months postoperatively compared to the PRF group and the Control group, with the PRF group also showing a significant improvement over the Control group (Figure 3, Table 4).

At 12 months post operation, in the HBO-PRF group of 50 cases, the number of cases with significant bone fill improvement was 47, which accounts for 94%, a proportion significantly higher than that of the PRF group (28%). The significant improvement rate (100%) was also significantly higher than that of the PRF group (88%) and the Control group (14%) (Table 5).

Comparison of the GPH and BTA: Among Groups at 12 months post operation, both the HBO-PRF group and the PRF group showed a significant increase in Gingival Papilla Height (GPH) and a significant reduction in Black Triangle Area (BTA) compared to the control group. The improvements in GPH and BTA in the HBO-PRF group were markedly better than those in the PRF group (Table 6).

Comparison of OPG and RANKL: Levels in GCF At 6 and 12 months post operation, the levels of Osteoprotegerin (OPG) in the Gingival Crevicular Fluid (GCF) of the HBO-PRF group were significantly higher compared to the PRF group and the Control group, while the levels of Receptor Activator of Nuclear Factor-kappa B Ligand (RANKL) and the RANKL/OPG ratio were significantly lower. The PRF group also showed significantly higher levels of OPG and significantly lower levels of RANKL and RANKL/OPG ratio at 6 and 12 months compared to the Control group (Table 7).

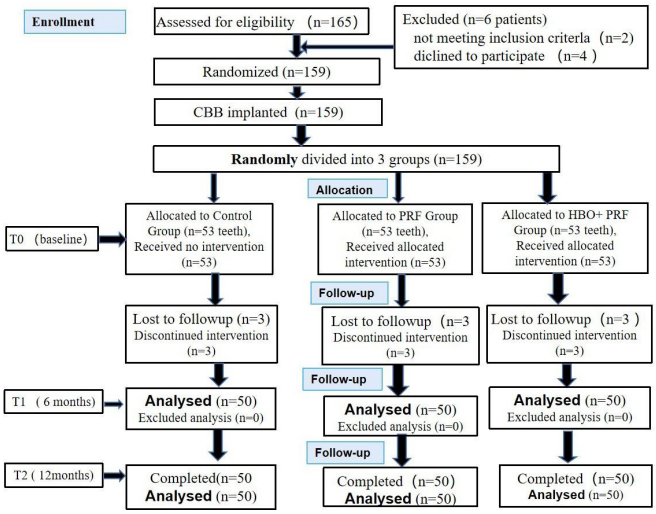

Figure 1: Study selection process.

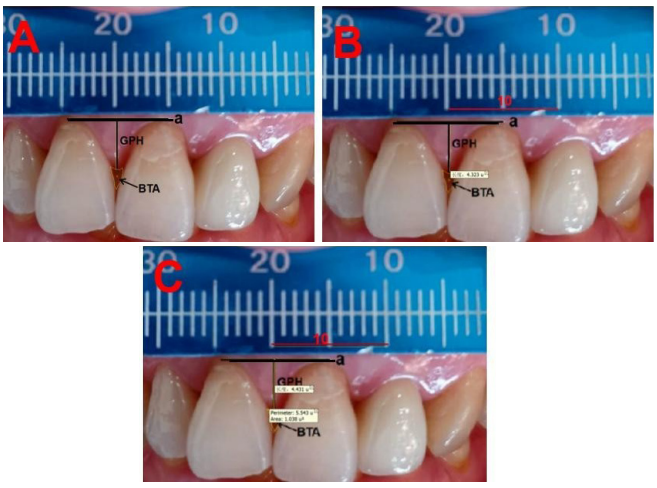

Figure 2: The photograph was processed by Digimizer 4.2 Image Program to determine GPH and BTA.
A: Measurement diagram of GPH and BTA.
B: Set the ruler to a scale of 10, and measure the GPA to be 4.323 u;
C: Set the ruler to a scale of 10, and measure the BTA to be 1.038 u^2; a: The line connecting the cemento-enamel junction on the labial surface of adjacent teeth. GPH: Indicating the gingival papilla height, BTA: Indicating the black triangles area.
Abbreviations: HBO: Hyperbaric Oxygen; PRF: Platelet Rich Fibrin; BTA: Black Triangles Area; GPH: Gingival Papilla Height, u: Units.

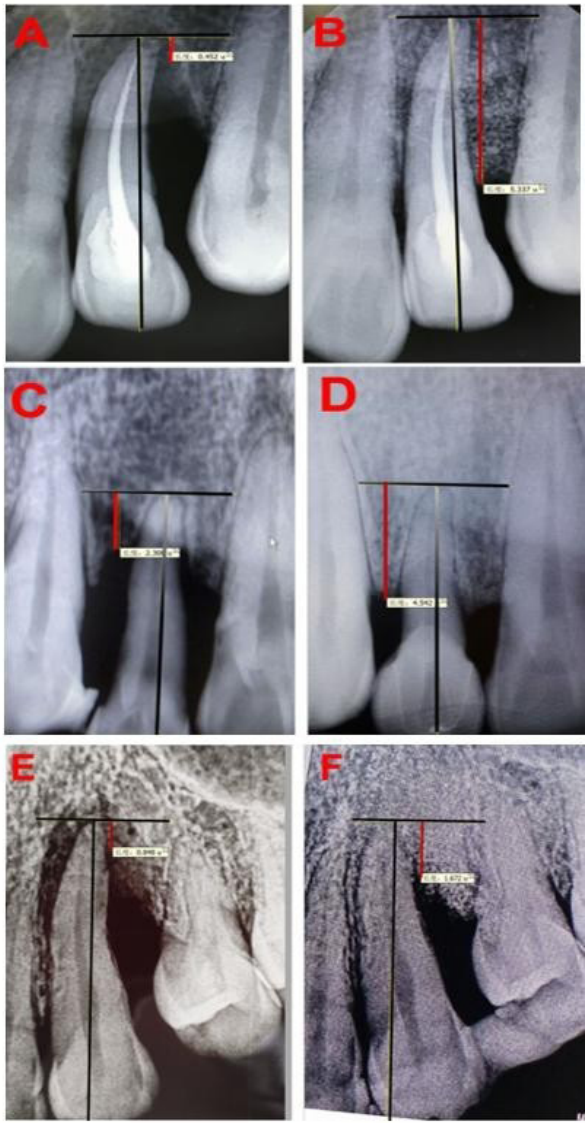

Figure 3: The photograph was processed by Digimizer 4.2 Image Program to determine gingival papilla height and black triangle area.
A: The periodontal condition of ⊥2 pre-therapy in the HBO-PRF group.
B: 12 months after treatment in the HBO-PRF group.
C: The periodontal condition of 2⊥ pre- therapy in the PRF group.
D: 12 months after treatment in the PRF group
E: The periodontal condition of⊥3 pre-therapy in the control group.
F: 12 months after treatment in thecontrol group.
Abbreviations: HBO: Hyperbaric Oxygen; PRF: Platelet Rich Fibrin.

Table 4: Comparison of the bone filling heights between groups at different time points (u).

Groups	Different time points		
	baseline	6M post-therapy	12M post-therapy
Control	1.15±0.01	1.83±0.08	1.71±0.10
PRF	1.22±0.02	3.48±0.10**	3.10±0.15*
HBO-PRF	1.25±0.01	5.35±0.15**##	5.12±0.13**##

Significant differences compared with Control group, *p<0.05, **p<0.01. Significant differences compared with PRF group, ## p<0.01.
♦ Numbers are mean ±standard deviations.
Abbreviations: HBO: Hyperbaric Oxygen; PRF: Platelet Rich Fibrin; M: month; U: Units.

Table 1: Comparisons of the GI, PD and AL between groups at baseline, 6 and 12 months.

Groups	n	GI	PD(mm)	AL(mm)
Control	50			
baseline		1.95 ± 0.30	6.60 ± 1.30	6.75 ± 1.10
6M post-therapy		0.93 ± 0.40**	3.81 ± 1.10**	5.25± 1.05*
12M post-therapy		1.05 ± 0.41**	3.78 ± 1.05**	5.35 ± 1.00*
PRF	50			
baseline		1.90 ± 0.42	6.51 ± 1.20	6.70 ± 1.30
6M post-therapy		0.65 ± 0.25**#	2.80 ± 1.00**#	3.20 ± 0.50**#
12M post-therapy		0.63 ± 0.15**#	2.71 ± 1.05**#	3.01 ± 0.65**#
HBO-PRF	50			
baseline		1.93 ± 0.33	6.53 ± 1.30	6.67 ± 1.30
6M post-therapy		0.35 ± 0.13**##$	2.05 ± 0.25**##$	2.60 ± 0.32**##$
12M post-therapy		0.33 ± 0.10**##$	1.88 ± 0.35**##$	2.50 ± 0.40**##$

Significant differences compared with baseline, *P<0.05，**P<0.01. Significant differences compared with Control group, #p<0.05, ##p<0.01. Significant differences compared with PRF group, $P<0.05.
♦ Numbers are mean ±standard deviations.
Abbreviations: HBO: Hyperbaric Oxygen; PRF: Platelet Rich Fibrin; M: Month.

Table 2: Comparison of the KGW changes between groups at different time points.

Groups	Different time points post-therapy				
	2 Week	1M	3M	6M	12M
Control	0.45±0.25	1.35±0.30	1.39±0.35	1.42±0.45	1.41±0.40
PRF	0.36±0.15	0.69±0.40*	0.82±0.30*$	0.83±0.40*$	0.84±0.25*$
HBO-PRF	0.25±0.10	0.45±0.15**#	0.59±0.15**#$	0.60±0.30**#$	0.61±0.15**#$

Significant differences compared with Control group, *p<0.05, **p<0.01. Significant differences compared with PRF group, #p<0.05. Significant differences compared with 1 month post-therap, $P<0.05.
♦ Numbers are mean ±standard deviations.
Abbreviations: HBO: Hyperbaric Oxygen; PRF: Platelet Rich Fibrin; M: Month.

Table 3: Comparison of the bone density between groups at different time points (HU).

Groups	Different time points		
	baseline	**6M post-therapy**	**12M post-therapy**
Control	130.10±10.50	601.50±25.30	580.60±15.40
PRF	133.20±15.10	669.60±17.40*	671.50±19.10*
HBO-PRF	125.45±12.50	725.50±30.25**#	734.40±15.3**#
	baseline	**6M post-therapy**	**12M post-therapy**
Control	130.10±10.50	601.50±25.30	580.60±15.40
PRF	133.20±15.10	669.60±17.40*	671.50±19.10*
HBO-PRF	125.45±12.50	725.50±30.25**#	734.40±15.3**#

Significant differences compared with Control group, *p<0.05, **p<0.01. Significant differences compared with PRF group, #p<0.05.
♦ Numbers are mean ±standard deviations.
Abbreviations: HBO: Hyperbaric Oxygen; PRF: Platelet Rich Fibrin; M: month.

Table 5: Comparison of the efficacy of bone filling improvement at 12 months between groups ♦.

Groups	Total number	Cases number of bone filling improvement at 12M post-therapy (%)		
		Mild improvement	Moderate improvement	Severe improvement
Control	50	43 (86%)	7 (14%)	0(0%)
PRF	50	6 (12%)	30 (60%)**	14(28%)**
HBO-PRF	50	0 (0%)	3 (6%)**##	47 (94%)**##

Significant differences compared with Control group, **p<0.01. Significant differences compared with PRF group, ##p<0.01.
Abbreviations: HBO: Hyperbaric Oxygen; PRF: Platelet Rich Fibrin; M: Month.

Table 6: Comparison of the GPH and BTA between groups at baseline and 12 months post-therapy ♦.

Groups	GPH (u)		BTA (u^2)	
	Baseline	12M post-therapy	Baseline	12M post-therapy
Control	3.41±0.25	3.37±0.30	1.25±0.13	1.28±0.19
PRF	3.48±0.30	3.79±0.28*	1.30±0.11	0.99±0.10*
HBO-PRF	3.40±0.25	4.10±0.20**#	1.24±0.10	0.87±0.11**##

Significant differences compared with Control group, *p<0.05, **p<0.01. Significant differences compared with PRF group, #p<0.05, ## p<0.01. ♦ Numbers are mean ±standard deviations.
Abbreviations: HBO: Hyperbaric Oxygen; PRF: Platelet Rich Fibrin; M: Month; GPH: Gingival Papilla Height; BTA: Black Triangle Area; U: Units.

Table 7: Comparison of the OPG, RANKL, RANKL/OPG in GCF between groups at different time points ♦.

Groups	n	RANKL(Pg/ul)	OPG(Pg/ul)	RANKL/OPG
Control	50			
baseline		341.25 ± 8.50	126.50 ± 3.30	2.71 ± 0.48
6M post-therapy		314.50 ±3.40**	155.21 ±5.60**	2.02± 0.20**
12M post-therapy		325.15 ±3.30	140.33 ± 4.15	2.32 ± 0.25*
PRF	50			
baseline		344.10 ±2.40	129.40 ± 1.20	2.66 ± 0.50
6M post-therapy		298.25 ± 2.20**#	172.70 ±4.20**#	1.73 ± 0.45*#
12M post-therapy		308.30 ±3.25*#	165.40 ±5.15*#	1.87 ± 0.35**#
HBO-PRF	50			
baseline		339.90 ±4.15	127.50 ± 2.10	2.67 ± 0.40
6M post-therapy		279.30 ± 3.10**##	198.15 ± 4.25**##	1.40± 0.20**##$
12M post-therapy		288.22 ±4.10*##	182.05 ±2.70**##	1.58 ± 0.15**##$

Significant differences compared with baseline, *P<0.05, **P<0.01. Significant differences compared with Control group, # p<0.05, ## p<0.01. Significant differences compared with PRF group, $ P <0.05. ♦ Numbers are mean ±standard deviations.
Abbreviations: HBO: Hyperbaric Oxygen; PRF: Platelet Rich Fibrin; M: Month; RANKL: Receptor Activator of Nuclear Factor Kb Ligand; OPG: Osteoprotegerin.

Discussion

Periodontal tissue regeneration has always been a challenging and hot topic in the treatment of periodontitis. PRF, as a second-generation autologous platelet concentrate containing cells, growth factors, and fibrin bio-scaffolds, has seen changes in its physicochemical properties due to the development of low-speed centrifugation techniques. These changes make PRF closer to the ideal tissue regeneration system in terms of bio-signaling molecules and bio-scaffolds, promoting vascular regeneration and tissue healing. The combined application of PRF with other technologies can better promote periodontal tissue regeneration. Because it is easy to obtain and cost-effective, PRF has been applied in the treatment of periodontal tissue regeneration in recent years [10]. Systematic reviews have shown that PRF is significantly effective in the treatment of intrabony periodontal defects, and the efficacy of PRF combined with flap surgery is better than that of bone grafting alone [4]. Since platelets in PRF can activate and release growth factors to stimulate the proliferation and differentiation of mesenchymal stem cells from bone marrow, promoting collagen synthesis, and the three-dimensional fibrin network structure is conducive to the migration of osteoblasts and new bone formation [11]. In vitro experiments have found that PRF can promote cell proliferation and differentiation, and the regeneration of hard and soft tissues, and platelets in PRF can slowly release growth factors for up to 28 days [12]. Animal experiments have confirmed that PRF combined with Periodontal Ligament Cells (PDLC) can promote periodontal tissue regeneration in rats [13]. Clinical studies have found that PRF combined with hydroxyapatite has a certain curative effect on human periodontal bone defects [14] and promotes the healing of periodontal bone defects [15]. Electron microscopy confirmed that the collagen fibers in PRF are arranged in a loose and porous network structure, containing a large number of stationary or pseudopodia-extended platelets and white blood cells located between the fibers [16]. This structure can promote vascular and tissue regeneration, and white blood cells have anti-infection effects, which are beneficial to tissue healing. The findings of this study show that the GI, PD, and AL in the PRF group were significantly improved at 6 and 12 months postoperatively compared to the control group, indicating that PRF has a significant effect on the clinical efficacy of periodontal treatment.

Regarding the role of PRF in gingival recession, Aroca S et al. [17] showed that PRF can improve the thickness of keratinized gingiva and the level of periodontal attachment in patients with gingival recession. At 12 months, the root coverage rate in the PRF group (76.6%) was similar to that of the connective tissue graft group (77.4%), indicating that PRF can be used as an alternative to connective tissue grafting in the treatment of gingival recession [18]. The present study found that at 1, 3, 6, and 12 months post operation, the changes of KGW in the PRF group were significantly less than those in the control group. The changes of KGW at 6 and 12 months were similar to those at 3 months, suggesting that the effect of PRF on KGW becomes stable by 3 months postoperatively. This is consistent with the theory that growth factors in PRF are continuously released.

Animal experiments have demonstrated that platelet-rich plasma (PRP) combined with bone graft materials can promote the repair of bone defects [19]. PRF combined with PDLC and mesenchymal stem cells (MSCs) facilitates the formation of periodontal tissue in nude rats [20]. The bFGF secreted by PRF, when combined with CBB, has good therapeutic effects on peri-

odontal bone regeneration [21]. The present study found that at 6 and 12 months postoperatively, the bone density in the PRF group was significantly better than that in the control group. At 12 months, the bone filling height in both the PRF group and the HBO-PRF group was significantly better than that in the control group, consistent with the results of Sharma A [22].

Although PRF (PRF) has shown good effects on periodontal bone defects, the difficulty in bone regeneration efficacy is due to the anaerobic bacterial infection and the anatomical characteristics of periodontitis. Therefore, the research on the combination of PRF with other technologies to promote the regeneration of periodontal tissue is of great significance. This clinical study adopted the treatment of periodontal bone defects with PRF combined with HBO. The results showed that at 6 and 12 months postoperatively, the GI, PD, and AL in the HBO-PRF group were significantly improved compared to the PRF group. The bone density, bone filling height showed significantly improved, which were better than those in the PRF group, and consistent with the results of Johannes [23]. The therapeutic effect of HBO is related to its ability to improve the osteogenic and angiogenic effects of bone marrow mesenchymal stem cells under inflammatory conditions in vitro [24]. In addition, it is also related to the increased expression of Runx2 by HBO, which promotes calcium salt deposition and accelerates the formation of new bone [25].

The recession of the gingival papilla leading to black triangles is a challenging issue in the diagnosis and treatment of periodontal diseases, and currently there is a lack of effective treatment methods. This study found that at 12 months postoperatively, both the HBO-PRF group and the PRF group had significantly higher GPH and significantly reduced BTA compared to the control group. This is related to the activation and release of a large number of growth factors and the fibrin scaffold structure by PRF [26]. The anti-inflammatory and tissue regeneration-promoting effects of HBO may have played a role in the postoperative regeneration of the gingival papilla [27-28]. These results are consistent with the findings reported by Awartani FA using hyaluronic acid gel treatment [29].

There are relatively few reports on the mechanisms by HBO promotes bone regeneration. Izumino J found that HBO has a therapeutic effect on rats with cranial defects because it promotes the expression of basic fibroblast growth factor (bFGF) in the early stages[30]. HBO can promote angiogenesis and the expression of vascular endothelial growth factor (VEGF) in animal bone defects, positively regulating bone healing [31]. In addition, HBO can stabilize and activate hypoxia-inducible factor 1 (HIF- 1), increase cell proliferation, and improve wound healing in animals [32].

Osteoprotegerin (OPG) and Receptor Activator of Nuclear Factor-kB Ligand (RANKL) are key cytokines that regulate bone metabolism. OPG inhibits the binding of RANKL to Receptor Activator of Nuclear Factor-kB (RANK) , reducing the activation and bone resorption of osteoclasts, while RANKL promotes the generation and activity of osteoclasts. The alveolar bone resorption in periodontitis is closely related to the RANK and its ligand RANKL and OPG [33]. Non-surgical periodontal treatment can reduce the level of RANKL in GCF, increase the expression of OPG, and significantly reduce the RANKL/OPG ratio and periodontal clinical parameters in GCF [34-35]. In addition, bovine lactoferrin can inhibit lipopolysaccharide induced periodontal bone destruction, downregulate the level of RANKL, and upregulate the level of OPG [36]. Animal experiments have found that the combination of HBO and bFGF has a synergistic effect on the healing of bone defects in rats, which is related to the increased expression of OPG and CD34 [37]. This study found that there were significant differences of RANKL, OPG, and the RANKL/OPG in GCF at 6 and 12 months between the HBO-PRF group and the control group. And there were also significant differences between the HBO-PRF group and the PRF group, indicating that the synergistic effect of HBO combing PRF on bone defects is related to the expression of OPG and RANKL. Further research with a larger sample size is needed to elucidate the detailed mechanism of action.

Conclusion

The combination of HBO and PRF can significantly improve the efficacy of CBB in repairing periodontal bone defects, enhance the level of periodontal attachment, promote periodontal bone regeneration, increase the KGW and the GPH, and improve black triangles. Its efficacy is associated with the capacity of HBO to elevate oxygen levels within the gingival tissue, regulate HIF-1α, and improve microcirculation, and the PRF is rich in growth factors and scaffold structures. The mechanism is related to its regulation of the RANKL/OPG signaling pathway, balancing the osteoclastic and osteoblastic processes, and affecting the expression of OPG and RANKL. This study provides a new and effective treatment method for the repair of periodontal bone defects and increase the KGW and improve gingival papillae recession.

Abbreviations: HBO: Hyperbaric Oxygen; PRF: Platelet-Rich Fibrin; CBB: Calcined Bovine Bone; GI: Gingival Index; PD: Probing Depth; AL: Attachment Level; CBCT: Cone-Beam Computed Tomography; ELISA: Enzyme-Linked Immunosorbent Assay; OPG: Osteoprotegerin; RANKL: Receptor Activator Of Nuclear Factor-Kb Ligand; GCF: Gingival Crevicular Fluid; KTW: Keratinized Gingival Width; PRP: Platelet-Rich Plasma; Bfgf: Basic Fibroblast Growth Factor; VEGF: Vascular Endothelial Growth Factor; HIF: Hypoxia-Inducible Factor; GPH: Gingival Papilla Height; BTA: Black Triangle Area; U: Units; M: Months.

Declarations

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