Comparative Study of advance platelet rich fibrin/concentrated growth factor technology in regenerative periodontal surgery: a randomized controlled clinical trial

I. background

Periodontitis is one of the most common oral diseases, which is characterized by alveolar bone resorption and destruction. Severe periodontitis has become the sixth largest epidemic disease in the world, and it is the leading cause and main cause of tooth loss in adults. As a major country in the incidence of periodontitis, there is a huge population of patients with severe periodontitis in China, and the incidence rate is as high as 12.1-16.1%. The ideal treatment of periodontitis is not only to eliminate pathogenic factors and control inflammation, but also to reconstruct and repair the damaged periodontal tissue structure and achieve periodontal tissue regeneration so as to preserve natural teeth for a long time.

At present, guided tissue regeneration (GTR) combined with bone grafting is the main method for clinical treatment of periodontal bone defects. This classical operation uses membranous materials to shield the faster growing gingival epithelial cells and connective tissue cells to create an effective closed space and time for the growth of periodontal ligament cells with regenerative potential. as a result, new cementum is formed on the root surface and periodontal ligament fibers are embedded, resulting in regenerative healing. At the same time, the bone graft material was implanted into the periodontal tissue defect area to maintain enough submembrane space to help stabilize the blood clot which is essential for

periodontal regeneration, and then guide the formation of autogenous bone tissue. However, it has been reported that although GTR can repair intraosseous defects to a certain extent, the results of periodontal tissue restoration are far from achieving the desired goals of doctors and patients. At the same time, according to the 2015 consensus of the American periodontal Association on the treatment of periodontal bone defect regeneration, the existing indications of regeneration surgery are limited to narrow and deep subosseous pockets, according to this requirement, the shape and depth of most bone defects do not have the conditions for regeneration surgery, or even if the regeneration operation is performed, the effect of the operation is very limited, and the affected teeth can only be extracted in the end.

In order to improve the effect of periodontal regeneration therapy, scholars try to combine platelet concentrate containing a variety of growth factors with bone grafts in periodontal regenerative surgery to improve the ability of local bone induction and tissue healing. Among them, the latest generation of autologous platelet concentrate, modified platelet-rich fibrin (APRF), contains rich autologous growth factors that promote cell proliferation and migration, which can promote new angiogenesis, wound healing and accelerate tissue remodeling. Studies have shown that gel-like APRF contains collagen fibers close to the physiological state, which can provide an effective three-dimensional scaffold for cell proliferation, as a natural vascularization-inducing component and immune response support. Among them, TGF- β, VEGF and other growth factors can

promote angiogenesis, inhibit osteoclasts, facilitate the migration and proliferation of gingival fibroblasts, and provide a good microenvironment for the repair and regeneration of traumatic tissue. On the other hand, in 2006, Sacco et al used inner wall coating test tube with silica, concentrated growth factor (CGF) was prepared after differential centrifugation. CGF uses differential centrifugation technology, so the fibrin clot obtained is larger than platelet-rich fibrin (PRF), and the content of fibrin is more. The three-dimensional fibrin network structure of CGF can be observed under scanning electron microscope, red blood cells and platelets are fixed in the fiber network, and the fiber of CGF is thicker and more regular than PRF. The contents and release rules of key growth factors in APRF/CGF were compared, and it was found that both APRF and CGF could stably release higher concentrations of growth factors PDGF- α β, TGF- β and VEGF, while APRF released more and more sustained growth factors than CGF in the later stage, suggesting that APRF may have better structure and function of promoting tissue repair and regeneration than CGF. However, there is no significant difference in the efficacy of APRF and CGF platelet concentrates in periodontal regeneration surgery, and there are some defects such as low level of evidence, small number of case samples, short observation period and so on. At present, there are still few studies on the application of the two in the treatment of clinical periodontal tissue regeneration, and there is no report on comparing the clinical effects of the two through randomized clinical controlled trials.

Based on this, this study compares the clinical application of GTR combined

with APRF/CGF and simple GTR in promoting periodontal bone defect regeneration through randomized clinical controlled trials, and intends to further explore the efficacy of the three methods in improving the amount of alveolar bone regeneration, so as to provide a certain basis for future clinical work.

II. The purpose of the clinical trial

The purpose of this study is to compare the clinical application of GTR combined with APRF/CGF and simple GTR in promoting periodontal bone defect regeneration, and to further explore the efficacy of the three methods in improving the amount of alveolar bone regeneration, so as to provide a basis for future clinical work.

III. Study design

This study is a single-center, simple random parallel group design clinical trial, each random group is allocated according to the proportion of 1:1:1.

IV. Clinical trial site

Responsibilities: Second Affiliated Hospital of Zhejiang University; Lihong Lei; Tel: 057187784576;

Address: No. 88 Jiefang Road, Shangcheng District, Hangzhou, Zhejiang Province; Zip code: 310009

Participation Unit: the Second Affiliated Hospital of Zhejiang University Medical College; person in charge: Raverid Red

V. Design of research

1. Inclusion criteria

(1) Age 18-80 years old; (2) a teeth choice: 6-8 weeks after the teeth after the periodic treatment of teeth, there is at least one site periodontal diagnosis depth (PD) ⩾ 5mm, looseness is less than II The degree or II-III degree will be loose after a fixed fixed, imaging evaluation of the vertical bone absorption of the teeth is greater than 3 mm osteogenesis, and the site is free of teeth surgical treatment, the patient has no obvious symptoms; (3) Patients have better compliance, and the bacterial plaque control is good after the basic treatment (all-in-law bleeding index and the mushroom index), can understand the purpose of testing, willing to cooperate with surgery and follow-up, voluntarily participate in the test and signed Informed consent.

2. Exclusion criteria

(1) In the past 6 months, chewing smokeless tobacco, smoking or suction cigar once a week or more, or smoking more than 20 / week (1 pack / week); (2) patients before blood Three months taking any drug or platelet counts that affect platelet function less than 200,000 / mm3; (3) patients have taken anti-epileptic drugs, antiplasia, antidepressants, sedatives, califiers, anti-depression, sedatives in the

first 1 month before surgery Inflammatory drug or daily analgesic; (4) patients with diabetes history or blood glucose detection abnormal (fasting blood glucose ⩾7mmol / L); (5) liver, renal function is abnormal (AST, Alt ⩾ 1.5 times ULN, creatinine ⩾ 1.5 times ULN); (6) has serious endocrine and metabolic diseases; (7) There are historical history of three high blood pressure; (8) History of osteoporosis; (9) History of autoimmune diseases; (10) Because of malignant tumors or other serious medical history, surgery should not be accepted or led to observation of teeth; (11) Pregnancy or lactating women; (12) Patients with anesthetic allergy; 13) Clinical or radiology shows that there are acute infections, root lesions, roots, severe eradications, dental bone rats, non-easy to abrasive, and osteoplasses (CEJ) or There is no treatment of untreated bilrels in the roots, the repair body is gains and / or in CEJ or the following repair edges are not in close; (14) A subject that can cause oral imaging inspection artifacts, such as: The study of teeth and its neighboring teeth are metal dentures, porcelain teeth; (15) a patient with root-stricken lesions;

3. Diagnostic criteria

（1）. Periodontal pocket > 3mm, and inflammation, gingival bleeding （2）. Adjacent clinical attachment loss > 1mm （3）. Periodontal pocket bleeding on probing （4）. Alveolar bone has horizontal or vertical resorption. The progress of the disease is slow, and the teeth are loosened or displaced in the late stage.

4. Criteria for withdrawing / terminating tests

（1）. Any unexpected time to break blindness （2）. Patients who were found to be sensitive to or rejected implants during / after operation. （3）. Problems in need of treatment that met the exclusion criteria. （4）. Patients with irresistible factors such as non-migration / death lost follow-up during the study period.

VI. Research methods

1. Randomized controlled scheme

A simple random method is used for random grouping. The specific method is as follows: first, a column number 1x 78 is established by Excel2013, and then the random number between 0 and 1 is generated by the other column, and then sorted by the largest to the smallest. The first 26 numbers are assigned to the GTR group, the middle 26 numbers are assigned to the APRF+GTR group, and the last 26 numbers are assigned to the CGF+GTR group. The subjects were divided into GTR group, APRF+GTR group and CGF+GTR group according to 1:1:1. (2) researcher A put the implant name (APRF or CGF or none) corresponding to the group mark into 78 Kraft paper envelopes with serial number 1: 78 and opaque according to the distribution order list. A gave the sealed envelope to B and no longer participated in the follow-up study. Researcher B is responsible for including the subjects according to the inclusion criteria, assigning the tested according to the order of seeing a doctor, and is responsible for supervising the operation process.

2. Blind design

Researcher A put the name of the implant corresponding to the group mark (APRF or CGF or none) into 78 Kraft paper envelopes with serial number 1x78 and opaque according to the assigned order list. A gave the sealed envelope to B and no longer participated in the follow-up study. Researcher B is responsible for including the subjects according to the inclusion criteria and assigning the number of the subjects according to the order of seeing a doctor. During the operation, researcher B will hand over the sealed envelope to the surgeon according to the number, supervise the operation process, and do not participate in other studies such as measurement. The survey researcher is responsible for measuring various indicators on a regular basis. Until the end of the experiment, the measurement researchers did not know how the subjects were grouped.

3. Treatment scheme

The newly diagnosed patients were recalled after 6-8 weeks of basic treatment to evaluate whether they met the inclusion exclusion criteria and confirm the operation site. For the patients considering inclusion, they agreed on the operation time one week later (female patients avoided menstruation). Oral health education was given to the patients again. One week later, the operative (V0), the informed consent form was signed by the patient, and then the researchers measured the baseline of the included patients, including (1) clinical periodontal index: probing depth (PD), clinical attachment level (CAL), mobility

(mobility), probing bleeding (BOP). (2) Imaging examination: the height of bone defect (IC), bone level (RBL) and the angle of intraosseous defect were measured by CBCT image, and the volume of intraosseous defect was measured after three-dimensional reconstruction of the affected teeth: all measurements were repeated twice to take the average value; before the actual measurement, all measurement researchers have passed the consistency test. Researcher A uses a random number table for random grouping and uses opaque sealed envelopes for distribution and concealment, and then hands over the envelopes to researcher B and no longer participates in the follow-up study. Researcher B numbered the enrolled patients 1-78 in order. Before the operation, researcher B handed over the sealed envelope to the itinerant nurse to open it, and both the patient and the patient were blind at this time. Since then, researcher B is responsible for supervising the operation. All the operations were performed by experienced surgeons. The surgical area was anesthetized before operation, intraoral and perioral routine disinfection was performed, and the operation was carried out in accordance with the principles of routine periodontal regeneration and aseptic operation.

If the patient was assigned to the APRF+GTR group, 20ml was collected with a blood collection needle from the superficial elbow vein immediately before operation and placed in two special glass centrifuges for APRF. Immediately after the blood collection was completed, the blood was symmetrically placed in the TR-18plu series centrifuge, and the 14min was centrifuged with 1500rpm at room

temperature. After centrifugation, the intravascular blood was divided into three layers: the upper layer was PPP, the middle was yellowish cylindrical gel-like APRF, and the bottom layer was erythrocyte layer. The 2 test tubes completed by centrifugation were placed vertically on the operating compartment table for backup (not tilted or inverted). Simplified gingival papilla retained incision cut full thickness flap and fully relaxed incision and (the flap should be extended 1-2 teeth to the proximal and distal part of the tooth, if the adjacent tooth is missing, the incision should be extended at least 5-10 mm to the area of edentulous teeth). Thoroughly remove the root calculus, plaque and granulation in the defect area with a Gracey curette and spoon, expose the intraosseous defect, cut one piece of APRF gel into 1-2mm particles and mix with Bio-oss, and the other piece is pressed into a 1mm film with special equipment; trim the Bio-gide collagen membrane according to the shape of the bone defect, the membrane should cover the edge of the defect at least 2mm. The mixture of APRF and Bio-oss was filled into the bone defect, the Bio-gide membrane was trimmed, and then the APRF membrane was covered with it; the gingival flap was restored to close the window with improved cross-horizontal mattress suture; periodontal plug agents were placed in the operation area.

If the patient was assigned to the CGF+GTR group, the patient immediately used a blood collection needle from the superficial elbow vein to collect blood about 20ml, which was placed in two special CGF centrifuges with silica coating on the inner wall of the centrifugal tube. Immediately after the blood collection

was completed, the symmetrical Medifuge centrifuge was selected, and the CGF procedure was selected and centrifuged for 13 minutes. The CGF centrifugation procedure is as follows: accelerate for 30 seconds, rise to 2700r/min, centrifuge for 2 minutes, then descend to 2400r/min, centrifuge for 4 minutes, accelerate to 2700r/min, centrifuge for 4 minutes, then descend to 3000r/min, centrifuge for 3 minutes, and decelerate to stop in the last 36 seconds. Finally, the blood in the test tube is divided into three layers: the top layer is PPP, the lowest layer is crimson jelly-like red blood cells and platelets, and the middle layer is yellow translucent gel-like CGF. The 2 test tubes completed by centrifugation were placed vertically on the operating compartment table for backup (not tilted or inverted). The APRF gel in the above process was changed to CGF gel, mixed and covered with CGF, and the other operations were the same.

If the patient was assigned to the GTR group, no blood was drawn. Gel or membrane containing growth factor was not used to cover the operation, and the rest of the operation was the same.

At the end of the operation, the surgeon gave the patient postoperative medical advice and given postoperative medication: cefuroxime axetil 50mg, ornidazole 500mg, twice a day for 5-7 days. Oral hygiene education was given to the patients again, and patients were advised to use Koutai mouthwash twice a day for 2 weeks, and about 2 weeks later to remove stitches. Two weeks after operation, stitches were removed by the original surgeon, and the patients were scheduled for 12 weeks ±7 days (V1), 24 weeks ±14 days (V2) and 48 weeks ±28

days (V3).

VII. Observation index and evaluation of curative effect

The patients were contacted and followed up by researcher B at 12, 24 and 48 weeks after operation. At 12 weeks ± 7 days after operation, PD, CAL, mobility and BOP were measured by measurement researchers. 24 weeks ±14 days and 48 weeks ±28 days after operation, the researchers measured PD, CAL, mobility, BOP, RBL, IC, intraosseous defect angle and bone defect volume.

PD（probe depth）:use a periodontal probe (UNC-15,Hu-Friedy,Chicago,IL) paralleling to the long axis of the tooth to measure the distance from the bottom of the periodontal pocket to the gingival margin (mm), record six sites of each tooth (the mesial, median and distal site of the buccal and tongue surface).

CAL (clinical attachment level) : The distance from periodontal pocket bottom to CEJ was measured by periodontal probe (mm). Record six sites of each tooth (the mesial, median and distal site of the buccal and tongue surface).

mobility: use a tweezer to clamp the incisal areas of the anterior teeth or the pit groove on the occlusal surface of the posterior teeth and shake the teeth faciolingually, mesiodistally and vertically. I degree : abnormal mobility faciolingually or less than 1mm positioning. II degree : abnormal mobility faciolingually and mesiodistally or loosening range of 1-2mm. III degree : abnormal mobility faciolingually, mesiodistally and vertically or loosening range is

greater than 2mm.

BOP(bleeding of probe) : observe for 10-15 seconds after probe to see whether it bleeds.Record six sites of each tooth (the mesial, median and distal site of the buccal and tongue surface).

RBL(radiographic bone level): Locate the reference point at the deepest PD site of the affected tooth: cemento-enamel junction (CEJ), alveolar crest (AC), bone defect (BD) .RBL is the straight line distance from CEJ to BD (mm)

IC: IC is the straight line distance from AC to BD (mm)

regenerated bone volume parameters : in mimics software, threshold was set according to the gray value of the affected tooth and its surrounding alveolar bone, image editing and region growth were used to form a mask of the three-dimensional reconstruction area. The three-dimensional calculation function was used to reconstruct the three-dimensional shape of the alveolar bone in the target area. Furthermore, the structure of alveolar bone was optimized by remeshing and smooth. The volume of alveolar bone before and after operation was calculated by Boolean operation to obtain the model of regenerated alveolar bone and its volume was measured.

Defect fill (%) = (preoperative IC- and postoperative IC) / preoperative IC × 100%

Defect resolution (%) = (preoperative RBL- and postoperative RBL) / preoperative RBL × 100%

VIII. Statistical analysis method (statistical software: SPSS25.0)

1) data description: measurement data are described by mean ±standard

deviation, non-normal distribution data are described by median and percentage (25th 75th percentile)

(2) baseline data are compared with normal distribution by one-way ANOVA test, while measurement data of non-normal distribution are described by Wilcoxon rank sum test.

(3) to compare the curative effect, first of all, the normality and homogeneity of variance of the experimental data were tested. If it obeys normality and homogeneity of variance, one-way analysis of variance (One-Way ANOVA) is used to compare the differences of mean among groups. If there were significant differences, pairwise comparisons were made, and multiple comparisons were made with SNK-q test to analyze and compare the differences between groups. If it does not obey normality and homogeneity of variance, Friedman test is used to compare the mean difference of each group; if there is significant difference, pairwise comparison is made by Wilcoxon Matched-Pairs Sighed Rank test. $P < 0.05$ was used as an index of statistical difference.