

## **Application of platelet rich fibrin combined with atorvastatin in the regeneration treatment of degree II furcation involvements of mandibular molars.**

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### **Abstract**

**Objective:** To study the application value of Platelet Rich Fibrin (PRF) combined with atorvastatin in the regeneration treatment of degree II furcation involvements of mandibular molars.

**Methods:** Thirty patients with periodontitis (30 cases of degree II furcation involvements of mandibular molars) who underwent surgical treatment in our hospital from June 2015 to June 2016 were randomly divided into study group and control group, with 15 cases in each group (15 cases of degree II furcation involvements of mandibular molars). Both of the two groups were treated with periodontal Guided Tissue Regeneration (GTR) combined with bone graft, in which the study group was plus treated with Platelet-Rich Fibrin (PRF) combined with atorvastatin, and the control group was plus treated with PRF. Cone Beam Computed Tomography (CBCT) was used to evaluate the curative effect before and 6 months after this operation. The plaque index, gingival index and changes in clinical attachment loss were observed. The changes of IL-6 and TNF- $\alpha$  concentration in gingival crevicular fluid before and 6 months after operation were compared between the two groups.

**Results:** There were no significant differences in plaque index, gingival index, clinical attachment loss, CBCT metrics and IL-6 and TNF- $\alpha$  contents in the gingival crevicular fluid between the two groups ( $P>0.05$ ) before the operation. Six months after operation, the plaque index, gingival index, clinical attachment loss, and IL-6 and TNF- $\alpha$  concentration in the gingival crevicular fluid in the study group were significantly lower than those in the control group ( $P<0.05$ ). Compared with the control group, the CBCT metrics, vertical bone loss and horizontal bone loss, in the study group were significantly improved at baseline ( $P<0.05$ ).

**Conclusion:** PRF combined with atorvastatin can effectively reduce the levels of IL-6 and TNF- $\alpha$  in gingival crevicular fluid and promote the effect of bone grafting in the treatment of degree II furcation involvements of mandibular molars, which is worth popularizing.

**Keywords:** Platelet-rich fibrin (PRF), Atorvastatin, Bone graft, Periodontitis, Mandibular molars, Furcation involvements.

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### **Introduction**

Periodontitis is one of common, frequently-occurring infectious and destructive diseases. If inflammation invades to the root furcation of the multirooted teeth, it will destroy the tissue at root furcation, causing pathological bone resorption, which is called furcation involvements [1]. The main purpose of periodontal therapy is to restore the patient's periodontal health and function. The conservative treatment and flap operation are designed to eliminate subgingival plaque. The tissue healing method in these treatments is long junctional epithelium, which cannot achieve true periodontal tissue regeneration [2]. Studies have shown that [3], for most single

rooted teeth, this standard treatment can achieve better efficacy, but once the root furcation of a molar is involvement, its response to traditional treatment will be different from the single rooted teeth. Due to its unique, complex anatomical structures and various responses to treatment, root furcation defects represent a difficult problem in a class of clinical therapies. Related studies show that [4], conservative treatment and resection surgery can only delay the progress of the attachment loss of furcation involvements, but cannot stop the loss. Therefore, for degree II furcation involvements, regeneration treatment must be taken as soon as possible where conditions permit.

At present, the most effective regeneration therapy for degree II furcation involvements is the combination of bone grafting and Guided Tissue Regeneration (GTR) [5]. GTR can promote the attachment of furcation involvements in the horizontal and vertical directions. Through the use of different mechanical barrier membranes, GTR can promote the completely cured rate of furcation involvements to 67% [6]. However, the therapeutic effect of GTR is still affected by the regeneration environment and the regeneration potential of the root furcation region. Therefore, its efficacy is not fully predictable. Recent studies [7] have applied growth factors such as enamel matrix proteins and basic fibroblast growth factor to the GTR and have achieved good therapeutic effects. However, there is no data on the effect of PRF combined with atorvastatin for GTR in the treatment of degree II furcation involvements of mandibular molars. The aim of this study was to evaluate the value of PRF combined with atorvastatin in the treatment of degree II furcation involvements of mandibular molars, in order to provide a theoretical reference for the clinical treatment of degree II furcation involvements of mandibular molars.

## Materials and Methods

### General information

Thirty cases of periodontitis patients (30 cases of degree II furcation involvements of mandibular molars) who were received treatment in our hospital from June 2015 to June 2016 were selected. There were 20 males and 10 females aged from 25 to 55 y old, with an average age of  $(38.3 \pm 2.5)$  y old). Inclusion criteria: The patients who were clinically diagnosed as chronic periodontitis, and were diagnosed as degree II furcation involvements by Hamp's classification; the patients whose periodontal pocket depth  $\geq 5$  mm and Horizontal Probing Depth (HPD)  $\geq 3$  mm after basic treatment for mandibular molars and the patients who needed periodontal surgery; radiology showed that there was a radiolucent region in the furcation region; venous blood platelet count was greater than  $100 \times 10^9/L$ ; the patients did not take any drug that had effect on platelet function 3 months before blood taken; the patients were informed consent. Exclusion criteria: the patients who had teeth with abnormal pulp vitality or who have received treatment of pulp; pregnant and lactating women; the patients who had liver, kidney and other vital organs diseases; in the root furcation region, the dental tissues had caries, defects or had been splitting; other non-operative indications; the patients who cannot cooperate with the completion of this experiment. The 30 patients were randomly divided into study group and control group, with 15 patients in each group (15 cases of degree II furcation involvements of mandibular molars). There was no significant difference between the two groups in gender, age and other general data. The study was approved by the Academy of Medical Ethics, and the patients were informed consent.

### Isolation and preparation of PRF

Half an hour before the operation, about 5 ml of vein blood was collected by a disposable vacuum blood collection needle to a sterile centrifuge tube without any anticoagulant. The tube was then immediately centrifuged at 3000 r/min for 12 min. The blood was separated to 3 layers, and the middle layer was the PRF gel. The preparation of PRF did not contain any additives such as anticoagulants. The blood began to agglutinate once it came into contact with the centrifuge tube. Therefore, the successful extraction of PRF requires rapid blood collection and immediate centrifugation. After centrifugation, the centrifuge tube was placed in a 37°C water bath.

### Surgical method

The two groups were treated with periodontal Guided Tissue Regeneration (GTR) combined with bone grafting: (1) Before the operation, the patients were guided to use 0.12% chlorhexidine solution to rinse mouth for 1min, and the patients received conventional disinfection, placement of towels, and local anesthesia. (2) An inverse bavel incision was made at the gingival margin of the surgical area, and the keratinized gum was retained as far as possible. (3) The full thickness flap was turned, and the defects 3-4 mm adjacent to bony cortex were exposed to facilitate the placement of the guided membrane. (4) The defect area was debrided, the granulation tissue was scraped completely, the root surface was flattened, and the alveolar bone was dressed. Minocycline was used to treat the root surface for 3 min, and normal saline was used to rinse the surface.

During the surgery, in the control group, the PRF was pressed into a membrane using sterile gauze, which was divided into two. One was prepared to fragments and mixed with bone particles to fill closely the bone defect. Then a GTR membrane of an appropriate size was placed at least 2-3 mm above the edge of the bone defect. The GTR membrane was required to fit around the edge to avoid folding. Then the GTR membrane was fixed to the teeth by sling suture. The other PRF membrane was placed under the surface of the GTR membrane. Then the gingival flap was sutured and applied with periodontal dressing.

**Study group:** Plus treated with atorvastatin drug on the basis of the control group: Atorvastatin calcium (Beijing Jialin Pharmaceutical Co., Ltd.), 20 mg/d, oral administration, for at least 6 months.

The GTR membrane was completely covered with the gingival flap in both groups to avoid the membrane exposure. The suture was removed 2 w after operation. Follow-up visits were carried out once every month in the first 3 months after operation, and once every three months later. No subgingival exploration was done 6 months after operation.

For intraoperative and 6-month postoperative clinical photos of case 7 in the study group (Figure 1). For 6-month postoperative CBCT presentation (Figure 2).

**Evaluation index**

(1) The preoperative and 6-month postoperative plaque index, gingival index, and clinical attachment loss of the patients in the two groups were compared and analysed. The clinical examination was performed and recorded by the same physicians who received regular training.

(2) CBCT images of the affected teeth were taken before and 6 months after operation, and measured by CBCT's own software i-Dixel-3DX, accurate to 0.01 mm. All measurements were made by the same experienced radiologist on the same monitor (19" Viewsonic VA703B monitor, Viewsonic, Walnut, US) in the darkroom, with the screen resolution of 1280 × 1024 pixels, at an interval of 1 week between two readings. Before the start of the study, the inspector's own consistency test was carried out [8]. CBCT measurement indicators were mainly the changes of vertical bone loss and horizontal bone loss.

(3) The changes of IL-6 and TNF-α contents in gingival crevicular fluid were measured by Enzyme-Linked Immunosorbent Assay (ELISA) before and 6 months after operation.

**Statistical analysis**

The data were analysed by the SPSS20.0 software. The changes of preoperative and postoperative clinical data and CBCT data between the two groups were compared by the paired t-test. The difference between the two groups was compared with the independent sample t-test. The difference was statistically significant when P<0.05.

**Results**

**Comparison of plaque index, gingival index, clinical attachment loss between two groups**

There were no significant differences in plaque index, gingival index and clinical attachment loss between the two groups

**Table 1.** Comparison of plaque index, gingival index, clinical attachment loss between two groups.

Group	Number cases (n)	Plaque index		Gingival index score (point)		Clinical attachment loss (mm)	
		Preoperative	6-month postoperative	Preoperative	6-month postoperative	Preoperative	6-month postoperative
Control group	15	4.26 ± 0.35	1.14 ± 0.16	4.77 ± 0.52	1.26 ± 0.08	4.58 ± 0.11	1.19 ± 0.05
Study group	15	4.28 ± 0.41	1.97 ± 0.22	4.79 ± 0.63	2.03 ± 0.15	4.56 ± 0.12	2.16 ± 0.07
T		-0.081	-5.293	-0.042	-7.845	0.213	-19.531
p		0.939	0.006	0.968	0.001	0.8422	0.000

**Table 2.** Comparison of changes of CBCT measurement index between the two groups.

Group	Number of cases (n)	Vertical bone loss (mm)	Horizontal bone loss (mm)
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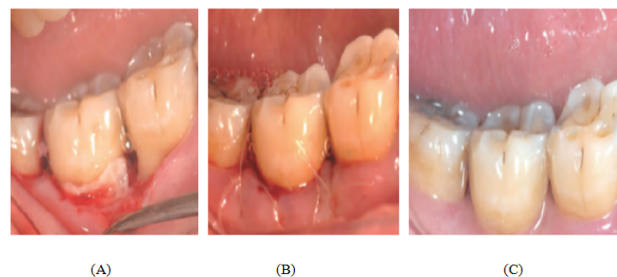
before operation (P>0.05). 6 months after operation, the plaque index, gingival index and clinical attachment loss were significantly lower in the study group than those in the control group (P>0.05, Table 1).

**Comparison of changes of CBCT measurement index between the two groups**

There was no significant difference of vertical bone loss and horizontal bone loss (CBCT measurement index) between the two groups before operation (P>0.05). 6 months after operation, the vertical bone loss and horizontal bone loss in the study group were significantly improved compared with those at baseline and those in the control group (P>0.05, Table 2).

**Comparison of IL-6 and TNF-α contents in gingival crevicular fluid between the two groups**

There were no significant differences in IL-6 and TNF-α contents in gingival crevicular fluid between the two groups before operation (P>0.05). 6 months after operation, the IL-6 and TNF-α contents in gingival crevicular fluid were significantly lower in the study group than those in the control group (P<0.05, Table 3).

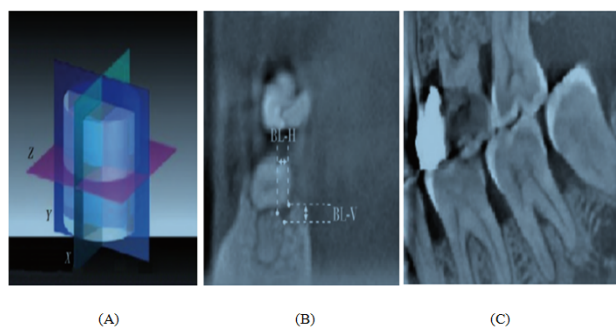


**Figure 1.** Intraoperative and postoperative clinical photos of case 7 in the study group. A. mixture in site and CGFs membrane in site; B. wound closed; C. 6 months post-surgery.

		Preoperative	6-month postoperative	Preoperative	6-month postoperative
Control group	15	5.17 ± 0.42	2.03 ± 0.34	5.32 ± 0.36	3.08 ± 0.15
Study group	15	5.16 ± 0.37	2.95 ± 0.46	5.29 ± 0.41	4.01 ± 0.36
t		0.053	-2.809	-0.107	-4.751
p		0.960	0.048	0.920	0.009

**Table 3.** Comparison of IL-6 and TNF- $\alpha$  contents in gingival crevicular fluid between the two groups.

Group	Number of cases (n)	IL-6 (pg/ml)		TNF- $\alpha$ (pg/ml)	
		Preoperative	6-month postoperative	Preoperative	6-month postoperative
Control group	15	10.32 ± 2.37	3.12 ± 0.56	7.65 ± 1.24	2.34 ± 0.25
Study group	15	10.38 ± 2.04	4.57 ± 0.31	7.67 ± 1.09	3.96 ± 0.44
t		-0.057	-3.878	-0.035	-5.394
p		0.957	0.018	0.973	0.011



**Figure 2.** CBCT presentation of case 7 in the study group. A. 3D sketch map of CBCT; B. X plane of CBCT (sagittal plane); C. Y plane of CBCT (coronal plane). BL-H, bone loss in the horizontal direction, the distance from furcation entrance to the deepest site of horizontal bone loss; BL-V, bone loss in the vertical direction, the distance from furcation entrance to the bottom of bone pocket.

## Discussion

Furcation involvements are one of the reasons that the molar loss is much higher than that of other teeth. The anatomical features are more complex in the root furcation region, thus determining the complexity of the treatment for furcation involvements [9]. How to find a highly effective treatment has been a hot spot in clinical research. Related data shows [10] that, the PRF growth factor, in the role of fibrin carrier, can be released slowly. Some studies observe the sustainable release of PRF for 28 d, which meets the time requirements for tissue regeneration; at the same time, with the functions of hemostasis and anti-infection, the PRF can promote epithelial healing and guide the vascular regeneration. Statins have anti-inflammatory effects. Atorvastatin is a relatively important statin. This drug can consume allogeneic pentadiene, thereby inhibiting interleukin-1, interleukin-6 mediated inflammatory response [11]. Isoprenoid precursors are one of the essential substances for post-translational lipid modification

(isopentene) and are one of the essential substances needed by Ras and guanosine triphosphate adenosine for functioning [12]. These guanosine triphosphate adenosine enzymes, such as Ras, Rho, Rac, are intracellular signaling proteins that are activated to participate in signal transduction from extracellular stimuli to receptor coupling in the cytoplasm and nucleus. Atorvastatin and other statins can reduce the production of inflammatory factors by blocking isoprenoid-induced intracellular signaling pathways [13]. The levels of inflammatory factors such as IL-6 and TNF- $\alpha$  in the gingival crevicular fluid are used as subclinical molecular markers for the progression and severity of periodontitis [14].

Our present results showed that there was no significant difference in plaque index, alveolar index, clinical attachment loss, IL-6 and TNF- $\alpha$  contents in gingival crevicular fluid between the two groups before operation ( $P > 0.05$ ). At 6 months after operation, the plaque index, gingival index and clinical attachment loss, IL-6 and TNF- $\alpha$  contents in gingival crevicular fluid were significantly lower in the study group than those in control group ( $P < 0.05$ ). It is suggested that PRF combined with atorvastatin can effectively improve the plaque index, gingival index, and clinical attachment loss and reduce the levels of inflammatory factors IL-6 and TNF- $\alpha$ . The reason may be that PRF combined with atorvastatin can produce synergistic effects, and continuously reduce the production of interleukin-6 and interleukin-8 mediated by interleukin-1 in endothelial cell lines.

At present, examination of furcation involvements and analysis of efficacy are mainly dependent on clinical examination and periapical film. In the clinical examination, the clinician uses the probe to explore the furcation region to determine the horizontal and vertical attachment loss in the furcation region [15]. However, the examination is affected by the level of clinicians, shapes of probes, probing forces, inflammation of the gums, lengths of posts, anatomy of teeth and many other factors, with relatively low accuracy. Furthermore, as an

important auxiliary tool, periapical film can only display two-dimensional images. On the one hand, it cannot reflect the horizontal condition of bone destruction in the furcation region; on the other hand, it is difficult to accurately show the actual situation of the bone defects due to the overlap of the buccal lingual bone plate. Furthermore, it cannot distinguish buccal lingual lesions [16]. Walter et al. [17] investigated the accuracy of CBCT in the analysis of furcation involvements of maxillary molars. They shoot the CBCT for 25 maxillary molars before flap surgery and compared the data obtained from CBCT with the data obtained by direct examination. The results showed that the complete agreement between the two was as high as 84%. The results of this study showed that there was no significant difference in the CBCT metrics of vertical bone loss and horizontal bone loss between the two groups before operation ( $P>0.05$ ). At 6 months after operation, the vertical bone loss and horizontal bone loss in the study group were significantly improved compared with those at baseline and in the baseline group ( $P<0.05$ ). These data suggested that PRF combined with atorvastatin can effectively improve the vertical bone loss and horizontal bone loss.

In summary, PRF combined with atorvastatin can effectively reduce the levels of IL-6 and TNF- $\alpha$  in gingival crevicular fluid and promote the treatment effect of bone grafting in degree II furcation involvements of mandibular molars, which is worth popularizing. The limitations of this study are that the sample size and observation time are limited. In the future, the effect of PRF combined with atorvastatin on furcation involvements and whole periodontal tissue regeneration can be evaluated by expanding the sample size and prolonging the observation time.

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