Abstract: This study was conducted to investigate the short-term effects of a self-ligating appliance for orthodontic treatment of severe adult periodontitis. Thirty patients diagnosed as severe periodontitis were recruited at Nanjing Stomatological Hospital, P. R. China, between January 2012 and January 2016. General clinical and demographic data were collected from the patients, all of whom were treated with a self-ligating appliance. Probing pocket depth (PPD), clinical attachment level (CAL), bleeding on probing (BOP) and plaque index (PI) were measured before appliance placement, and at 1 and 3 months after appliance placement, respectively. Results showed the rate of tooth loss, mean PPD, mean CAL and the BOP ratio were more favorable in healthy subjects than in the patients. Smokers accounted for a significantly higher proportion of the patients in comparison with the healthy subjects. Clinical outcomes revealed that both the mean PPD and mean CAL were significantly decreased compared with the baseline ($P < 0.05$). Furthermore, the percentage of BOP, PI and bone mineral density were also significantly decreased at 1 month after treatment ($P < 0.05$). The volume of gingival crevicular fluid, as well as the levels of alkaline phosphatase, aspartate aminotransferase and glutathione peroxidase, were significantly increased in the first month after treatment, being decreased at 2 months, and finally returning to normal in the third month. In summary, orthodontic treatment using a self-ligating appliance can apparently improve the periodontal condition of patients with severe adult periodontitis.

Keywords: self-ligating appliance; orthodontic treatment; severe periodontitis.

Introduction

Periodontitis is a chronic inflammatory disease characterized by progressive destruction of tooth-supporting tissues, having a multifactorial etiology that includes interactions among specific bacteria, a host response and risk factors (1). It is induced predominantly by gram-negative bacteria, and influenced by a complex biofilm in the subgingival microenvironment, social and behavioral modulations, and host-related genetic or epigenetic traits (2). Diagnosis of periodontitis is based on a number of clinical symptoms, including swollen or discolored gums, bleeding on brushing, increased spacing between the teeth, loose teeth, pus between the teeth and gums, unpleasant intraoral taste, and halitosis (3).

Between 1990 and 2010, the global age-standardized prevalence of severe periodontitis was static at 11.2% (4). In 2012, according to global periodontal disease epidemiology, 5-20% of any population suffer from severe periodontitis, and in these populations, mild to moderate periodontitis affects a majority of adults (5). An epidemiological study of periodontitis in the United
States that involved a sample of 3,742 adults >30 years old revealed that over 47% of them, representing 64.7 million adults, had periodontitis, being severe in 8.5% of the total. Moreover, among adults ≥65 years old, 64% had either moderate or severe periodontitis (6). In addition, some studies have reported that severe periodontitis is associated with a number of cardiovascular problems, such as subclinical atherosclerosis, in young otherwise systemically healthy patients (7).

Periodontal-orthodontic treatment, which is usually performed to correct mal occlusions and may also improve periodontal health, is now widely used in the treatment of severe periodontitis (8). This form of treatment commonly employs fixed appliances such as brackets, bands, or fixed retentions. One of the major goals of periodontal-orthodontic treatment is to change the predominantly pathogenic microbiota to one that is host-compatible, and to achieve microbiological stability (9). However, orthodontic appliances and accessories may be harmful to oral hygiene, leading to accumulation of dental plaque and gingival inflammation, particularly in the posterior segments of the oral cavity (10,11).

Recently, the self-ligating approach has been attracting attention in the field of lingual orthodontics. Studies have suggested that self-ligating appliances have two major advantages compared with traditional ligature vestibular systems: chair-time reduction, and reduction of unwanted frictional forces (12-14). Although the clinical application of self-ligating appliances has become more popular, the influence of such appliances on periodontal tissues during periodontal-orthodontic treatment remains unclear. The present study investigated the short-term effects of a self-ligating appliance on periodontal tissues during periodontal-orthodontic treatment for severe adult periodontitis.

**Materials and Methods**

**Patients**

The present prospective study included a total of 30 patients diagnosed as having severe periodontitis at Nanjing Stomatological Hospital, P. R. China, between January 2012 and January 2016. The mean age of these patients was 40.41 ± 5.22 (range: 25-55) years and the male:female ratio was 16:14. Patients with periodontitis were included in the study when their periodontal pockets had a probing pocket depth (PPD) of >5 mm and the marginal alveolar bone loss was >30% for ≥50% of their teeth (15,16). Patients were excluded if they had medical disorders such as cancer, type-2 diabetes, hypertension, major cardiovascular/endocrine diseases, and pregnancy, lactation, or systemic conditions. Periodontal disease was defined as aggressive or chronic according to the American Academy of Periodontology (1999) (17). Smoking habits were investigated through a questionnaire. In addition, 20 healthy subjects were recruited as a control group to compare their clinical parameters with the study patients. Periodontal therapy was considered to have been effective when periodontitis had become non-active. Basic clinical information on the patients is shown in Table 1. The present study was approved by the Ethics Committee of Nanjing Stomatological Hospital (2015NL-016[KS]), and written informed consent was obtained from all patients.

**Treatment and clinical monitoring**

All subjects were treated with a self-ligating appliance (AO and ORMCO, Orange, CA, USA). They were instructed to maintain good oral hygiene during the treatment, and were provided with professional oral cleaning and oral hygiene instructions on toothbrushing. Interdental cleaning devices were also provided four weeks before the orthodontic treatment. A removable retainer was used when the fixed appliances were removed.

Periodontal clinical measurements were performed at six points per tooth for all teeth. PPD and clinical attachment level (CAL) were measured using the Florida electronic pressure-sensitive periodontal probe system (Florida Probe, Gainesville, FL, USA). Bleeding on probing (BOP) and plaque index (PI) were also recorded. Duplicate measurements were conducted to ensure a standard deviation of <1.0 mm, and the mean of the measurements for all of the examined teeth was recorded. These measurements were taken at three time points: T1 = just before appliance placement, T2 = 1 month after appliance placement, and T3 = 3 months after appliance placement.

**Measurement of bone mineral density**

Alveolar bone mineral density was measured using cone beam computed tomography (CBCT; NewTom VG, Verona, Italy). The exposure volume was set at a height of 30 mm and a width of 40 mm. The tube voltage was 80 kV with a current of 5 mA. The CBCT unit and its software were regularly calibrated in accordance with the manufacturer’s instructions. For measurement of alveolar bone mineral density, briefly, the median sagittal plane of the tested teeth as well as the other two planes with distance of 0.25 mm to the median sagittal plane were chosen (Fig. 1). On each plane, bone density was measured on the tongue (palatine) side of the alveolar ridge area, the middle root zone, and the apical area. The mean value of the three planes was defined as the...
mean value of the bone density. The measurements were conducted before and 3 months after treatment.

Collection and measurement of gingival crevicular fluid (GCF)
Both the mesial and distal sites were gently dried for 5 s and isolated from saliva with a cotton roll before GCF collection. GCF was collected from every site using a periopaper strip (Periopaper, Oraflow Inc., Plainview, NY, USA). The strip was carefully inserted for 60 s into the gingival crevice and left in situ for 60 s. Then, the samples were placed into separate sealed tubes, and immediately frozen at −80°C. GCF on the strip was recollected using the centrifugal washing method, and then thawed at 4°C. Phosphate buffer solution was added, and the mixture was shaken for 1 h, followed by centrifugation using a high-speed centrifuge (1,000 r/min) for 10 min. The liquid supernatant was collected for analysis using a Beckman Automatic Chemical Analyzer (Beckman Coulter Commercial Enterprise Co., Ltd., Beijing, P. R. China). Alkaline phosphatase (ALP), aspartate aminotransferase (AST) and glutathione peroxidase (GSH-Px) content and the volume of GCF were recorded before and at 1, 2, and 3 months after treatment, respectively.

Statistical analysis
The measurement data was expressed as mean ± standard deviation (SD). Independent continuous variables were compared using t-test and categorical data were compared using chi-squared test or Fisher’s exact test. Differences at $P < 0.05$ were considered statistically significant. All analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA).

Results
Basic clinical parameters
The clinical parameters are shown in Table 1. Sixty percent of patients were identified as having aggressive periodontitis. The percentage of smokers among patients (60%) was significantly higher than among healthy subjects (30%) ($P < 0.05$), indicating that smoking might be a risk factor for periodontitis. Furthermore, the percentage of patients who had tooth loss and the ratio of BOP among the patients were significantly higher than among the healthy subjects ($P < 0.05$). Both the mean PPD and mean CAL were significantly higher in the patients than in the controls ($P < 0.05$). Meanwhile, the levels of cholesterol, HDL, LDL and triglycerides, and the incidence of a family history of cardiovascular disease did not differ significantly between the two

| Clinical parameters | Patients $n = 30$ | Healthy people $n = 20$ |
|---------------------|------------------|------------------------|
| age (range)         | 40.41 ± 5.22     | 41.12 ± 7.23           |
| (25-55)             | (28-59)          |
| male: female        | 16:14            | 10:10                  |
| smokers, never, (n%)| 12 (40)*         | 14 (70)                |
| aggressive periodontitis (n%) | 18 (60)         | –                      |
| tooth loss          | 2.51 ± 0.44*     | 0.40 ± 0.12            |
| mean PPD (mm)       | 5.12 ± 1.13*     | 1.82 ± 0.56            |
| mean CAL (mm)       | 4.97 ± 1.05*     | 1.75 ± 0.41            |
| bleeding on probing | 50.45 ± 12.14*   | 12.36 ± 4.18           |
| cholesterol, mmol/L | 4.81 ± 1.45      | 4.79 ± 1.23            |
| HDL, mmol/L         | 1.42 ± 0.85      | 1.48 ± 0.76            |
| LDL, mmol/L         | 2.73 ± 1.12      | 2.85 ± 1.03            |
| triglycerides, mmol/L | 1.51 ± 0.31    | 1.57 ± 0.49            |
| family history of cardiovascular disease | 4 (13.3%) | 2 (10%) |

* $P < 0.05$ compared with the healthy group. PPD: probing pocket depth; CAL: clinical attachment level; HDL: high density lipoprotein; LDL: low density lipoprotein.
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Mean PPD, mean CAL, BOP and bone mineral density before and after treatment
The mean PPD, mean CAL, BOP, PI and bone mineral density before and after treatment are shown in Table 2. Both the mean PPD and mean CAL decreased significantly relative to the baseline \((P < 0.05)\). Furthermore, the percentage of BOP, PI and bone mineral density were also decreased significantly at 1 month after treatment \((P < 0.05)\) (Table 2).

GCF parameters before and after treatment
Table 3 demonstrates the values of GCF parameters before and after treatment. Trends in the alteration of all evaluated indices (GCF volume, ALP, AST and GSH-Px) were similar. GCF volume, as well as ALP, AST and GSH-Px content, increased significantly in the first month after treatment, were decreased at 2 months, and then finally returned to the normal levels in the third month (Table 3).

Discussion
Periodontitis is a common dental disease that can lead to long-term chronic infection of periodontal tissues, repeated inflammation, resorption and destruction of alveolar bone, and finally result in tooth loss. The periodontitis morbidity rate increases with age, and around 8-30% of adults are diagnosed with periodontitis worldwide as reported in 2006 (18). Severe periodontitis is defined as a CAL of 5 mm (19), which can lead to or aggravate diseases such as diabetes, cardiovascular disease, pulmonary disease and obesity (20).

Periodontal non-surgical treatment, such as scaling and root planning, is conventionally employed for periodontitis, but has certain limitations. Recently, with improvements in periodontal disease therapeutics, orthodontic treatment has become popular, and tends to be chosen by patients with more severe periodontitis in order to align and level the dentition and improve occlusal relationships (21). Fixed appliances are currently the most common form of orthodontic treatment. However, traditional appliances may hamper the oral self-cleaning effect, thereby being detrimental to oral hygiene. Self-ligation has advantages over traditional methods in that it can reduce the time in the chair and minimize unwanted frictional forces, making it an attractive fixed appliance that is being increasingly investigated (22,23). Although many studies have investigated self-ligating appliances, few have focused on changes in the periodontal tissues of the orthodontic tooth during treatment, and this was the goal of the present study, which compared the general clinical data between patients with severe periodontitis and healthy subjects. It was found that the rate of tooth loss, mean PPD, mean CAL and the ratio of BOP were more favorable in the latter than in the former. Moreover, the proportion of smokers among the patients was significantly higher than that among the controls, confirming that smoking may be a risk factor for periodontitis, as suggested in previous studies (7,16,24). It has also been shown that the plasma levels of cholesterol and triglycerides are associated with periodontal disease (25). In the present study, however, the differences in cholesterol, HDL, LDL, triglycerides and family history of cardiovascular disease between the two groups were not statistically significant, possibly because of the small sample size.

Examination of clinical outcomes revealed that alterations in mean PPD, mean CAL, BOP, PI and bone mineral density after treatment were all significant, suggesting
that the treatment had a positive effect, in agreement with previous studies (26).

It has been suggested that systemic bone mineral density has some relationship with periodontal disease (27). Hsu et al. (28) reported that the density of bone around the teeth was significantly reduced after application of orthodontic force for 7 months. In the present study, a decrease in bone mineral density was also observed after treatment. Therefore, the orthodontic treatment may have had a restorative effect on pathological bone mineral density (29). It has also been suggested that the distribution of bone mineral density is changed after orthodontic treatment due to active bone remodeling during maturation (30). Moreover, Sultan et al. (31) have revealed that bone mineral density is related to inter-proximal CAL, an issue that warrants further investigation.

GCF is a transudate that penetrates into the gingival sulcus from the sulcus epithelium and gingival connective tissues. It has been shown that GCF contains mainly the metabolites of microorganisms present in gum plaque, and the breakdown products of host epithelial and connective tissues (32). Accordingly, analysis of substances present in GCF has been widely used to assess the condition of periodontal tissues. Perinetti et al. (33) reported that ALP in GCF may be applicable as a non-invasive clinical biomarker of skeletal maturation in periodontally healthy subjects scheduled for orthodontic treatment. It has also been revealed that ALP activity in GCF steadily increases within three weeks after the leveling and alignment stage of orthodontic treatment (34). Yan et al. (35) investigated the AST level in GCF during orthodontic therapy with a self-ligating appliance, and found that the AST level increased in the first month and decreased to a normal level at 3 months after treatment. Furthermore, the GSH-Px level was also thought to be closely correlated with the periodontal environment (36). Interestingly, in the present study, the levels of ALP, AST and GSH-Px all demonstrated similar trends, increasing in the first month and decreasing to normal after 3 months. Veberiene et al. (37) reported that AST activity in the pulp of mechanically loaded teeth showed no significant difference between patients after 6 months of orthodontic alignment and individuals who did not receive orthodontic treatment. The present results suggest that recovery of the AST level in patients receiving orthodontic treatment may occur sooner. Furthermore, the measured volume of GCF was similar to that in previous studies, providing new information about long-term alterations in this setting (38).

The present study had certain limitations. First, it examined only the short-term effects of a self-ligating appliance in adult patients with severe periodontitis. Second, the study was mainly descriptive in nature, and further insights will require more detailed investigations. Finally, real-time PCR was not used to test the quantity and quality of periodontal pathogenic bacteria before the start of orthodontic treatment.

In conclusion, the present study has investigated the effects of a self-ligating appliance on periodontal tissues after orthodontic treatment for severe adult periodontitis, focusing mainly on comparison of periodontal conditions with those before the treatment. Although the study was limited by a small sample size and involved a single ethnic group, some useful information was obtained in the context of treatment for periodontal disease.

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Conflict of interest

The authors have no conflict of interest to declare.

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