Metabolism by peptidases plays an important role in modulating the levels of biologically active neuropeptides. One of these neuropeptides, substance P (SP), a component of gingival crevicular fluid (GCF), may exponentiate the inflammatory process during orthodontic tooth movement. The aim of this study was to investigate the GCF levels of SP in patients using different bracket systems. Subjects were 10 patients (four males, six females; mean age, 25.1 ± 4.4 years) undergoing orthodontic movement (leveling) in the maxilla. Conventional brackets were placed on the left side, while the teeth on the right received self-ligating brackets. The teeth on the mandibular left side without any orthodontic attachments served as controls. GCF was sampled at 0, 1, 24, and 168 hours after initiation of treatment. Prevention of plaque-induced inflammation allowed assessment of the dynamics of mechanically stimulated SP levels in the GCF, which was determined using commercially enzyme-linked immunosorbent assay (ELISA) kits. GCF levels of SP for the Damon System sites were significantly lower than for the teeth with conventional brackets at 24 hours. This result indicates that the Damon System inhibited an increase in the amount of SP in the GCF. Thus, the Damon System is useful to reduce the inflammation and pain resulting from orthodontic forces. World J Orthod 2009;10:141–146.
was to better understand the mechanisms for converting physical stress to the cellular responses that occur during tooth movements. To monitor the expression of biologically active substances in humans in a noninvasive manner, changes in the composition of gingival crevicular fluid (GCF) during orthodontic tooth movement were studied. These aforementioned biologically active substances are produced by periodontal ligament cells and then diffusely excreted in sufficient quantities into the GCF.

However, little information is available concerning the effect of self-ligating brackets on SP in the GCF during orthodontic tooth movement in humans. In the present study, the levels of SP in the GCF alternately using Damon and conventional brackets were investigated at 0, 1, 24, and 168 hours after the initiation of orthodontic treatment using commercially enzyme-linked immunoabsorbent assay (ELISA) kits.

MATERIALS AND METHODS

Subjects

Ten adult orthodontic patients (four males, six females; mean age, 25.1 ± 4.4 years) were enrolled in this study after meeting the following criteria: (1) good general health; (2) lack of antibiotic therapy during the previous 6 months; (3) absence of anti-inflammatory drug administration in the month preceding this study; (4) healthy periodontal tissues with generalized probing depths < 3 mm and no radiographic evidence of periodontal bone loss; and (5) only slight crowding (< 3 mm) throughout the dental arch. Informed consent was obtained from all subjects after an explanation of the study protocol, which was reviewed by the ethics committee of Nihon University School of Dentistry at Matsudo, Chiba, Japan.

Experimental design

On the maxillary left side, conventional brackets (Spirit, 0.022-in slot; Ormco Japan, Tokyo, Japan) were placed, whereas on the maxillary right side, self-ligating brackets (Damon 3, Ormco Japan) were bonded (Fig 1). The teeth on the mandibular left side without any attachments served as controls. Leveling was carried out by a 0.014-in copper Ni-Ti wire (Ormco Japan).

Sulcus-probing depth, presence of plaque, and bleeding on probing were evaluated on both sides of the dental arch, as well as in the control teeth. Thereafter, GCF samples were collected at 0, 1, 24, and 168 hours after initiation of tooth movement.

GCF collection

GCF was collected from both sides and the control site at the same time using the method of Offenbacher et al° (Fig 2). Initially, all teeth were washed gently with water; the sites under study were isolated with cotton rolls (to minimize saliva
contamination) and air-dried. Paper strips (Periopaper, Harco, Tustin, California, USA) were carefully inserted 1 mm into the gingival crevice and allowed to remain there for 1 minute, after which a second strip was placed at the same site. Care was taken to avoid mechanical injury.

The volume of GCF on each paper strip was measured using a Periotron 8000 (Harco) calibrated with human serum. All paper strips were stored at –30°C until further processing. Protein concentrations in the extracts were estimated by the method of Bradford with bovine serum albumin used as the standard.

**Enzyme immunoassay**

SP level was measured twice using a commercial ELISA kit (Quantikine, R&D Systems, Minneapolis, Minnesota, USA), with the results expressed as pg/µg of total protein in the GCF.

**Statistical methods**

Statistical analysis between the groups was performed using one-way analysis of variance (ANOVA) and the Scheffé test. The level of significance was set at $P < .05$.

### RESULTS

**Clinical parameters**

There was no significant difference in the mean volume of GCF at any time point among the conventional bracket group (0.40 ± 0.06 µL), the self-ligating bracket group (0.39 ± 0.07 µL), and the control site (0.40 ± 0.05 µL) as, for example, shown for all three canines (Table 1). In all patients, plaque accumulation was minimal throughout the entire study and periodontal health was excellent, with no gingival bleeding. Further, probing depths remained less than 2 mm at all times.

At 24 hours, SP concentration in the canine with the self-ligating bracket was significantly lower than in the canine with a conventional bracket. The control canine shows an SP amount significantly lower than that of the experimental canines (Fig 3). However, there were no significant differences between the experimental sites and the control site at 0, 1, and 168 hours. The SP concentration at 24 hours in all other teeth was similar to that of the canines (Fig 4).

### DISCUSSION

In the present study, the mean volume of GCF collected from the groups was not significantly different (Table 1), which coincides with the results of Yamaguchi et al. The mean SP values for the experimental teeth were significantly higher than those for the control site after 24 hours in both groups, though SP

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**Table 1 Total volumes in the GCF from canines during orthodontic tooth movement (µL)**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Spirit (conventional)</th>
<th>Damon 3 (self-ligating)</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.40 ± 0.04</td>
<td>0.39 ± 0.06</td>
<td>0.40 ± 0.06</td>
</tr>
<tr>
<td>1</td>
<td>0.41 ± 0.07</td>
<td>0.41 ± 0.09</td>
<td>0.39 ± 0.07</td>
</tr>
<tr>
<td>24</td>
<td>0.42 ± 0.07</td>
<td>0.38 ± 0.08</td>
<td>0.41 ± 0.05</td>
</tr>
<tr>
<td>168</td>
<td>0.38 ± 0.05</td>
<td>0.40 ± 0.07</td>
<td>0.40 ± 0.08</td>
</tr>
</tbody>
</table>

There was no significant difference in the mean volume of GCF at any time point among the conventional bracket group, the self-ligating bracket group, and the control teeth in the canine. The results of GCF volume in other teeth were similar.
concentration in the teeth with self-ligating brackets was significantly lower than in those with conventional brackets. By 168 hours, however, the levels of SP had returned to approximately baseline levels (Fig 3). Further, it becomes obvious that the results of all teeth were similar to those of the canines (Fig 4).

Previous studies demonstrated that GCF levels of prostaglandin (PG) E₂ and proinflammatory cytokines, such as interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α, increased during human orthodontic tooth movement.12,13 Giannopoulou et al reported that initial orthodontic tooth movement induced pain and a rapid release of biochemical mediators such as PGE₂, IL-1β, and SP in the GCF.14 Luthman et al suggested that SP plays an important role in the pathogenesis of periodontitis. Thus, SP levels were significantly elevated in the GCF of disease-affected teeth as compared to healthy sites.16 Furthermore, Hanioka et al reported that SP showed significant correlation with IL-1β host response in GCF from patients with periodontitis.17

Fig 3 Changes in SP concentration in the GCF samples of the canines from conventional brackets, self-ligating brackets, and the control site at various time periods. Control = control teeth; Spirit = conventional brackets; Damon 3 = self-ligating brackets. Significant differences in concentrations are indicated by * (P < .001); significant differences between Spirit and Damon 3 are indicated by # (P < .001).

Fig 4 SP concentrations in the GCF samples from conventional brackets, self-ligating brackets, and control teeth at 24 hours. Significant differences between the control teeth and Spirit or Damon 3 are indicated by * (P < .001); significant differences between Spirit and Damon 3 are indicated by # (P < .001).
A recent study by Yamaguchi et al has shown that the levels of SP and IL-1β in the GCF are increased by orthodontic tooth movements. This finding suggests that SP participates in the complex network of mediators that regulate inflammation.

Furstman and Bernick suggested that periodontal pain is caused by pressure, followed by ischemia, inflammation, and edema. Further, Burstone differentiated between immediate and delayed pain response, with the former related to the initial compression of the periodontal ligament immediately after placement of an archwire. The latter pain develops a few hours later and is caused by an increased sensitivity of the nerve fibers to noxious stimuli, such as prostaglandins and histamines. Also SP, a neuropeptide released from nociceptors in the region of tissue damage, plays a role. It increases the firing rate of neurons that relay nociceptive information.

Erdinç and Dinçer reported that perception of pain during orthodontic treatment with fixed appliances reached a peak at 24 hours and decreased by day 3, which suggests that the perception of pain may be linked to the release of SP. The study by Yamaguchi et al also demonstrated that the levels of SP increased during orthodontic tooth movement compared with those of the control sites after 24 hours. Also, PGE2 and proinflammatory cytokines play an important role in the pathogenesis of periodontal inflammation during orthodontic tooth movement in humans. Thus, it seemed appropriate to investigate GCF levels of these inflammatory mediators using different bracket systems.

Eberting et al found that patients treated with Damon self-ligating brackets had significantly lower treatment times, required significantly fewer appointments, and had significantly higher American Board of Orthodontics scores than those treated with conventional edgewise brackets.

**CONCLUSION**

This study showed that using the Damon System decreases the amounts of SP in the GCF after the first 24 hours. All in all, this suggests that the Damon System may be useful in reducing inflammation and pain in response to mechanical orthodontic forces.

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