

## Comparisons of chondroitin sulphate levels in orthodontically moved canines and clinical outcomes between two different force patterns

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### ABSTRACT

**Purposes:** The aims were to monitor remaining interrupted force magnitudes, and to compare chondroitin sulphate (CS) levels in gingival crevicular fluid (GCF) of moved mandibular canines, rates of space closure and patients' pain and discomfort between interrupted and continuous orthodontic force patterns.

**Materials and method:** Fifteen Class I malocclusion patients (5 males and 10 females; aged  $17.00 \pm 3.18$  years) who required orthodontic treatment with first premolar extractions, were recruited. Interrupted force pattern was generated by elastomeric chains, and continuous force pattern by Nickel-Titanium closed coil springs. Initial force magnitude was 120 g. Elastomeric chains were replaced by new ones at the end of fourth week during the loaded periods. During the unloaded and the loaded periods, remaining interrupted force magnitudes were measured, and those of continuous force pattern were calibrated and controlled. GCF samples were collected with Periopaper® strips. CS levels were measured by competitive ELISA with WF6 monoclonal antibody during the 8-week control, the unloaded and the 8-week loaded periods. Rates of space closure were measured, and amount of pain and discomfort was assessed by visual analog scale (VAS) scores.

**Results:** Medians of interrupted force magnitudes were 120.0, 60.0, 50.0, 37.5 and 25.0 g, and after elastomeric chain replacement were 120.0, 62.5, 37.5, 25.0 and 25.0 g respectively. There were no significant differences in the median CS levels between the 8-week control and the unloaded periods, and between right and left mandibular canines. Medians of CS levels during the loaded periods, both interrupted and continuous force patterns, were significantly greater than those during the unloaded period ( $P=0.008$  and  $P=0.027$  respectively). Differences between medians of CS levels of interrupted and continuous force patterns during each 1-week loaded period were not significant. There was no significant difference in the rates of space closure, and the patients' pain and discomfort between interrupted and continuous force patterns.

**Conclusion:** Both interrupted and continuous force patterns, with 120 g initial force magnitude, cause no difference in biochemically-assessed bone remodeling activity, same rate of space closure and same patients' pain and discomfort. Initial orthodontic force magnitude, of both interrupted and continuous force patterns, may play an important role for alveolar bone remodeling and clinical outcomes.

**Key words:** Chondroitin sulphate, Gingival crevicular fluid, Interrupted force, Continuous force

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### INTRODUCTION

Force-generating materials such as elastomeric chains (interrupted force pattern) and Nickel-Titanium closed coil springs (continuous force pattern) are normally used for canine retraction. Elastomeric chains, delivering interrupted force pattern, must be replaced during orthodontic treatment, are effective for orthodontic tooth movement and have cost-benefit reason for being used in clinical orthodontic practice.<sup>1</sup> Nickel-Titanium alloy's characteristics are super-elasticity and shape memory effect, so Nickel-Titanium closed coil springs can express long range of light and continuous force during orthodontic tooth movement.<sup>2,3</sup>

Some studies compared efficiency of interrupted orthodontic force pattern generated by elastomeric chains to that of continuous force pattern generated by Nickel-Titanium closed coil springs during orthodontic tooth movement.<sup>1,4-7</sup> Leethanakul and colleagues used interleukin-1 $\beta$  and interleukin-8 levels in human gingival crevicular fluid (GCF) during orthodontic tooth movement as biochemical markers for evaluating effects of either interrupted or continuous force pattern, and concluded that continuous force pattern generated by Nickel-Titanium closed coil springs gave higher rate of canine movement, which correlated with interleukin-1 $\beta$  and interleukin-8 levels.<sup>7</sup>

Within 24 hours after initial orthodontic loading especially at compression sites, cellular response includes release of cytokines and/or growth factors that triggers biological processes relating to alveolar bone remodeling.<sup>7,8</sup> In our previous study<sup>9</sup>, we applied our patented WF6 monoclonal

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antibody, raised against the WF6 catabolic epitope of CS<sup>10</sup>, to compare CS levels in GCF of orthodontically moved maxillary canines between two different force magnitudes, and proposed that 70 g of continuous retraction force should be more suitable than 120 g continuous retraction force, for maxillary canine movement, because there was no difference in biochemically assessed bone remodeling activity, same rate of tooth movement, reduced pain, better comfort and less tooth tipping. We then speculated that initial orthodontic force magnitude, in both interrupted and continuous force patterns, might bring about similar alveolar bone remodeling and clinical outcomes. The aims of this study were, therefore, to monitor remaining interrupted force magnitudes, and to compare CS (WF6 epitope) levels in GCF of orthodontically moved mandibular canines, rates of space closure and patients' pain and discomfort between interrupted and continuous orthodontic force patterns.

## **MATERIALS AND METHODS**

### **Subjects**

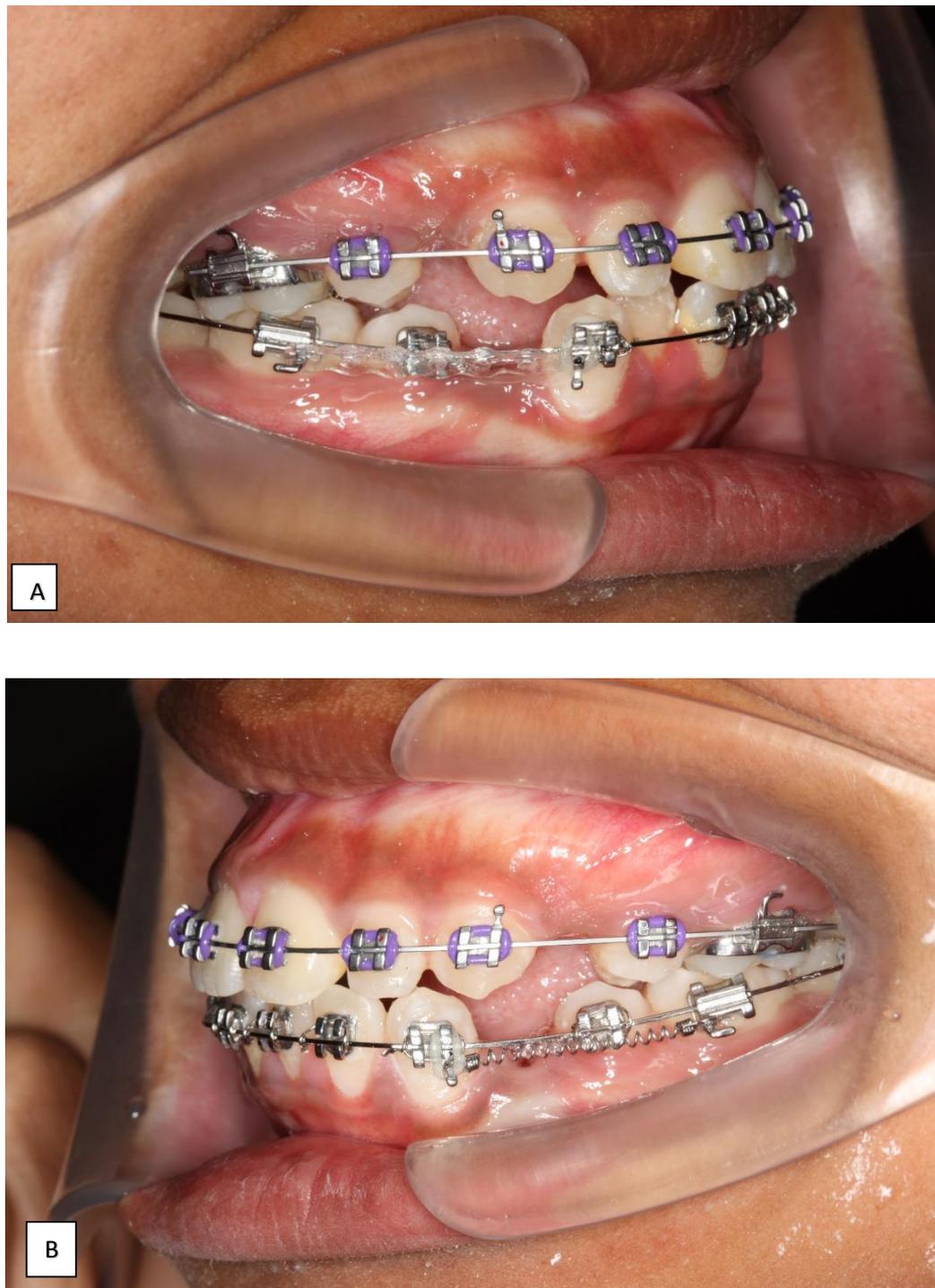
Fifteen patients (5 males and 10 females; aged  $17.00 \pm 3.18$  years; ranged from 11.97 to 22.92 years) were recruited. These patients met following criteria: (1) good general and oral health; (2) lack of antibiotic therapy during previous 6 months; (3) absence of anti-inflammatory drug administration in the month preceding the study; (4) no pregnancy (women); and (5) Class I malocclusion that required orthodontic treatment with first premolar extraction and distal canine movement. All patients received repeated oral hygiene instruction, and gingival health was controlled and maintained throughout the entire study. This study was approved by the Human Experimentation Committee of the Faculty of

Dentistry, Chiang Mai University. Informed consent was obtained from all subjects.

### **Experimental Design**

During the 8-week control period, GCF samples from right and left mandibular canines were collected with Periopaper® strips (ProFlow Inc., Amittyville, New York, USA) as control data. Orthodontic pre-adjusted brackets (Roth prescription slot 0.018" x 0.025") (3M Unitek Inc., Monrovia, California, USA) were bonded on mandibular teeth 3 weeks after first premolar extractions.

Prior to loading, at the beginning of first week during the 8-week loaded period, GCF samples of right and left mandibular canines were collected with Periopaper® strips as baseline data. During the 8-week loaded (experimental) period, the right mandibular canines were moved by Dynaflex® elastomeric chains (Dynaflex company, St. Louis, Missouri, USA), and the left mandibular canines by Nickel-Titanium closed coil springs (GAC, Central Islip, NY, USA). Initial orthodontic force magnitudes were calibrated at 120 g, for both interrupted (right) and continuous (left) force patterns, to move the mandibular canines distally on 0.016 x 0.016 inch stainless steel wire (Fig. 1). Elastomeric chains were replaced by new ones at the end of fourth and eighth weeks. Remaining interrupted force magnitudes generated by elastomeric chains was measured, at the end of each week from first to eighth week during the loaded period, by a force strain gauge (Dentaurum, Ispringen, Germany). Continuous force magnitudes generated by Nickel-Titanium closed coil springs were also calibrated and controlled at 120 g at the end of each week from first to eighth week during the 8-week loaded period. GCF samples from right and left mandibular canines were then collected with Periopaper® strips during the 8-week loaded period as experimental data.



**Figure 1** The right (A) and the left (B) experimental mandibular canines were retracted using either elastomeric chains (interrupted force pattern; 120 g initial force magnitude) or Nickel-Titanium closed coil spring (continuous force pattern; 120 g initial force magnitude).

**GCF Collection**

GCF collection was conducted as described previously.<sup>9</sup> Briefly, the teeth were gently washed, and isolated with a cotton roll. Then, supragingival plaque was removed without touching the marginal gingiva, and the crevicular site was gently dried with an air syringe. GCF was collected using 10.0x1.0 mm Periopaper®

strips (ProFlow™, Amityville, NY, USA) placed into the distal gingival sulcus of the mandibular canine until light resistance was felt, and left in the sulcus for 30 seconds. Care was taken to avoid mechanical injury to periodontal tissue. Strips contaminated with blood were discarded. Immediately after collection, the last 2.0 mm of

Periopaper® strip containing the GCF sample was cut off, individually transferred to microcentrifuge tubes, and individually stored at -80°C until further processing. An analytical instrument (Periotron 8000™, Oralflow Inc., Plainview, NY, USA) was used to measure the GCF volume. The volume of the GCF collected from the last 2.0 mm of each Periopaper® strip was averagely 0.01 µl.<sup>11</sup> To recover the CS biomolecules from Periopaper® strips, addition of 200-µl quantity of phosphate-buffered saline (pH 7.4) was performed, and the tube was then vigorously shaken for a few minutes at room temperature. The recovery rate (approximately 98 %) from each strip was determined by a dye-binding assay, using known concentrations of sulphated GAGs as standards.<sup>12</sup>

#### **Determination of the distance of tooth movement**

The study casts were made prior to and after orthodontic mandibular canine movement every 4 weeks until the 12<sup>th</sup> week in order to obtain a clearer picture of naturally slow tooth movement. The distance of mandibular canine movement was measured by using an ABSOLUTE digimatic caliper (Mitutoyo Corporation, Kawasaki, Japan). The measurement, which measured the maximum distance from the cusp tip of an orthodontically moved mandibular canine to the buccal groove of the first permanent mandibular molar, was performed.<sup>1</sup> The rate of space closure in millimeters (mm) per month was then calculated. To assess intra-examiner reliability and error of the method, the study models were re-measured by the same investigator 1 week later. The measurements were compared to the initial measurements using a paired t-test. There was no statistically significant difference between these two measurements.

#### **Evaluation for the amount of pain and discomfort**

Visual analog scale (VAS) was used to evaluate patient's pain and discomfort during orthodontic mandibular canine retraction. The patients reported their pain experience separately for each force pattern using the VAS at the end of first and fifth weeks during the loaded period. The linear scale properties ranged from 0 (Absence of pain) to 10 (Worst possible or unbearable pain).

#### **Competitive inhibition ELISA with WF6 monoclonal antibody**

The quantitative ELISA to determine the WF6 epitope of CS was performed using a protocol described previously.<sup>10</sup> In brief, microtiter plates (Maxisorp®, Nunc, Roskilde, Denmark) were coated overnight at room temperature with 10 µg/mL shark PG-A<sub>1</sub> fraction (100 µl/well) in coating buffer (20 mM sodium carbonate buffer, pH 9.6). On the following morning, the plates were washed three times with Tris-IB 150 µl/well and dried. Bovine serum albumin (BSA) 1% (w/v) 150 µl/well in incubating buffer (Tris-IB) was added to all plates. The plates were incubated at

37°C for 60 minutes to block non-specific adsorption of other proteins to the plates and washed. After washing, 100 µl/well of the mixture, samples or standard competitors (Shark PG-A<sub>1</sub>D<sub>1</sub> fraction: range 39.06-10,000 ng/mL) in mAb against the WF6 epitope (1:100) were added for 60 minutes at 37°C. Subsequently, the plates were washed, and the IgM-specific peroxidase-conjugated anti-mouse immunoglobulin (100µl/well; 1:2,000) was added and incubated at 37°C for 60 minutes. Then, the plates were washed and the peroxidase substrate (100 µl/well) was added and incubated at 37°C for 20 minutes to allow the color to develop. The reactions were stopped by the addition of 50 µl/well of 4M H<sub>2</sub>SO<sub>4</sub>. The absorbance ratio at 492:690 nm was measured using a Titertek Multiskan® MCC/340 multiplate reader (ICN/Flow Laboratories, Costa Mesa, California, USA). The minimal detection level of ELISA for CS was 0.019 ng/ml.

#### **Protein Assay**

Total protein concentration was determined by using the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, California, USA), based on the Bradford dye-binding procedure, a simple colorimetric assay for measuring total protein concentration. The known concentrations (0-1,000 µg/µL/well) of BSA standards and the GCF samples were added to the microtiter plates (10 µL/well) in triplicate. A mixture between dye reagent and de-ionized distilled water at 1:4 was added to each well (200 µL/well). The plates were incubated at room temperature for five minutes and the absorbance was measured at 620 nm. Protein concentrations were determined from a standard curve of BSA standards.

#### **Statistical analysis**

The data were analyzed using the Statistical Package for Social Sciences version 17.0 for Windows (SPSS Inc., Chicago, Illinois, USA). The Kolmogorov-Smirnov test was used to determine the distribution of CS (WF6 epitope) levels, the rates of space closure and VAS scores. The differences between the CS levels by either interrupted or continuous force pattern at each week during the 8-week control period and the unloaded period (baseline data) were compared using the Friedman's test. The differences between the CS levels at the unloaded period (baseline data) and during the 8-week loaded period (experimental data) were compared using the Wilcoxon signed-rank test. The differences between the two CS levels of force pattern (interrupted and continuous), as well as VAS scores, during each experimental period were compared using the Mann-Whitney U-test. The differences between the mean rates of space closure with interrupted force pattern and those with continuous force pattern were determined by the Independent-T-test. The results were considered statistically significant at  $P < 0.05$ .

**RESULTS**

**Force generated by either elastomeric chains or Nickel-Titanium closed coil spring during the 8-week loaded period**

The medians of forces generated by elastomeric chains at the beginning of first week to the end of fourth week were 120.0, 60.0, 50.0, 37.5 and 25.0 g, respectively. Then, after elastomeric chain

replacement, the medians of forces generated by elastomeric chains at the beginning of fifth week to the end of eighth week were 120.0, 62.5, 37.5, 25.0 and 25.0 g, respectively. Force generated by Nickel-Titanium closed coil springs at the beginning of first week to the end of eighth week was calibrated and controlled at 120 g (Fig. 2).

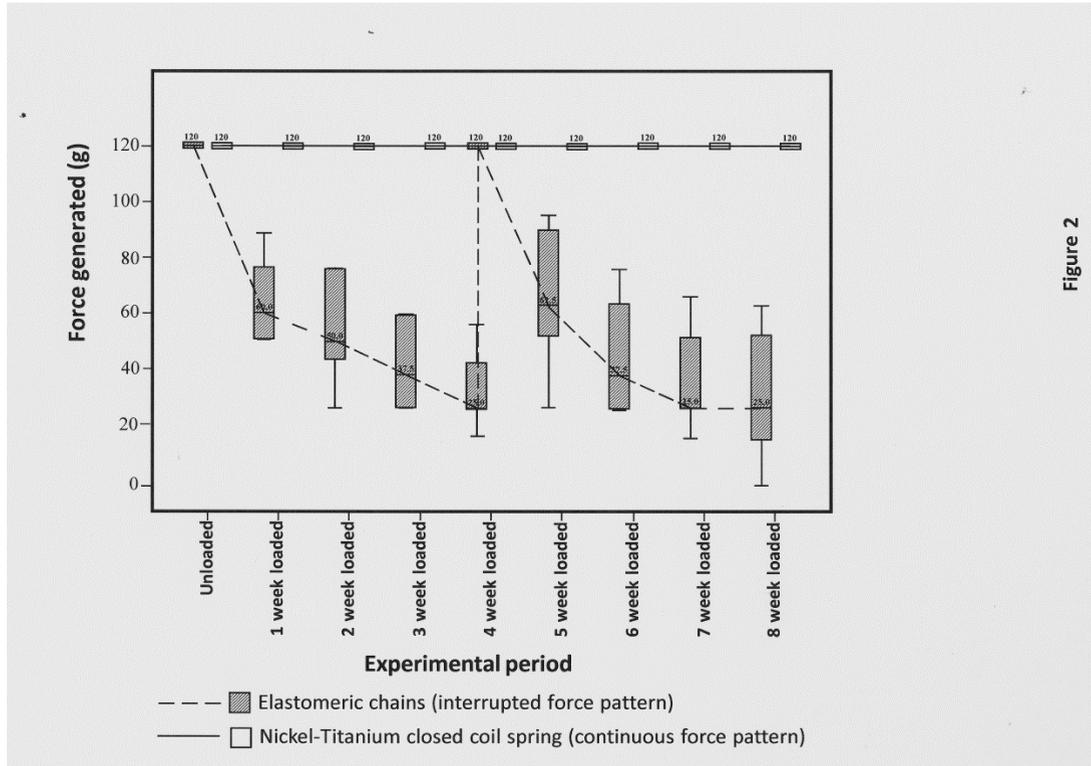


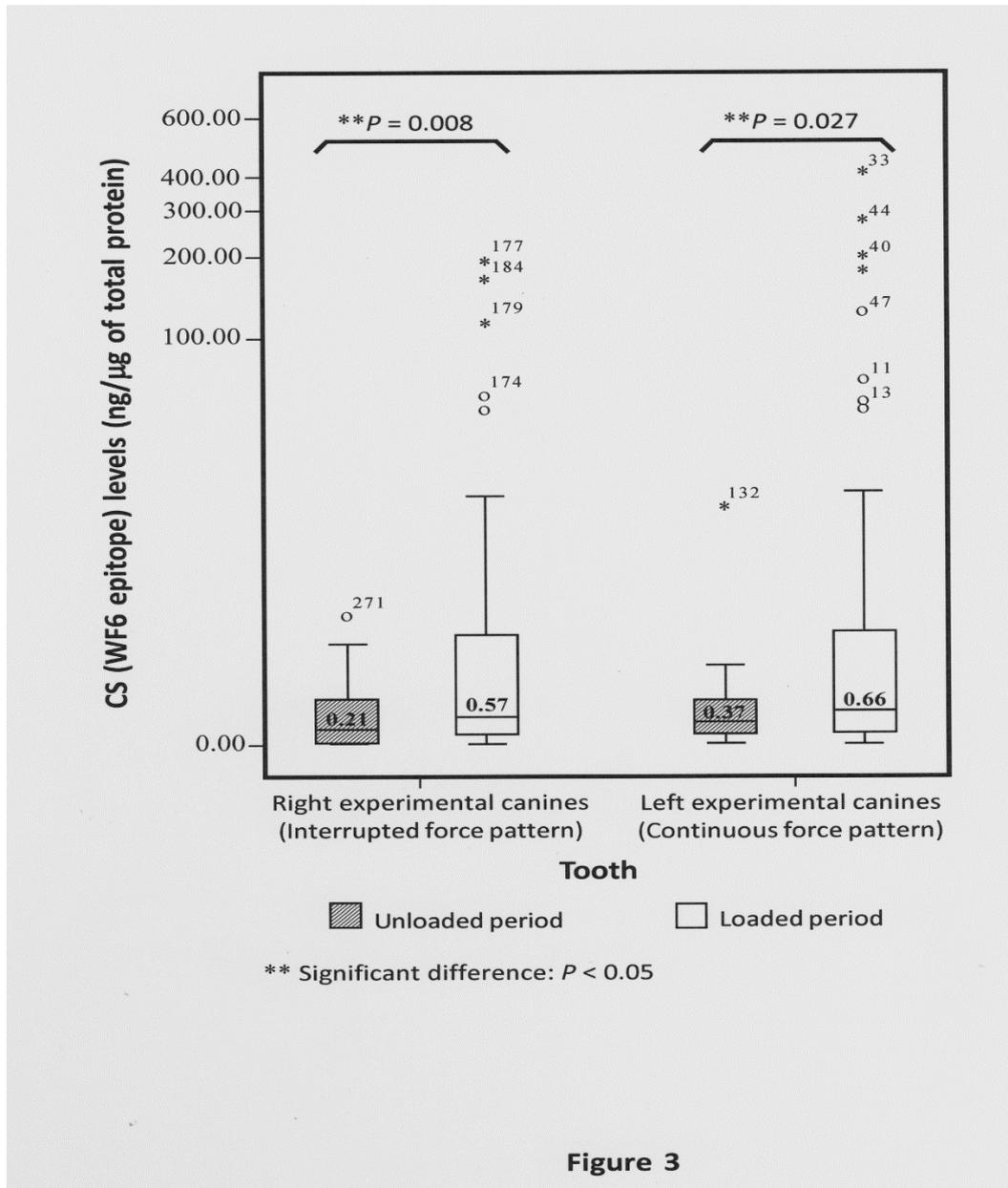
Figure 2

**Fig. 2** Boxplot graph of forces generated by either elastomeric chains (interrupted force pattern; 120 g initial force magnitude) or Nickel-Titanium closed coil spring (continuous force pattern; 120 g initial force magnitude) during the unloaded and the 8-week loaded periods

**Elevated levels of CS (WF6 epitope) in GCF of orthodontically moved mandibular canines**

During the 8-week loaded period (experimental data), the medians of CS levels around the right (interrupted force pattern) and the left (continuous force pattern) mandibular canines were 0.57 and 0.66 mg of total protein, respectively, and were significantly greater than those during the unloaded period (baseline data)

( $P=0.008$  and  $P=0.027$  respectively) (Fig. 3). Graphs depicting the profile of CS levels around the right (interrupted force pattern) and the left (continuous force pattern) mandibular canines, from one of our subjects, during the control, the unloaded (baseline) and the loaded (experimental) periods are shown in Fig. 4.



**Fig. 3** Boxplot graph of the chondroitin sulphate (CS; WF6 epitope) levels around the right and left experimental mandibular canines during the unloaded and the loaded periods (with interrupted and continuous force patterns). The boxes represent the values from 25<sup>th</sup> to the 75<sup>th</sup> percentile. The middle lines represent the medians. The vertical lines extend from the minimal to the maximal values, excluding the outlier marked with small open circles. The small asterisks represent the extreme values.

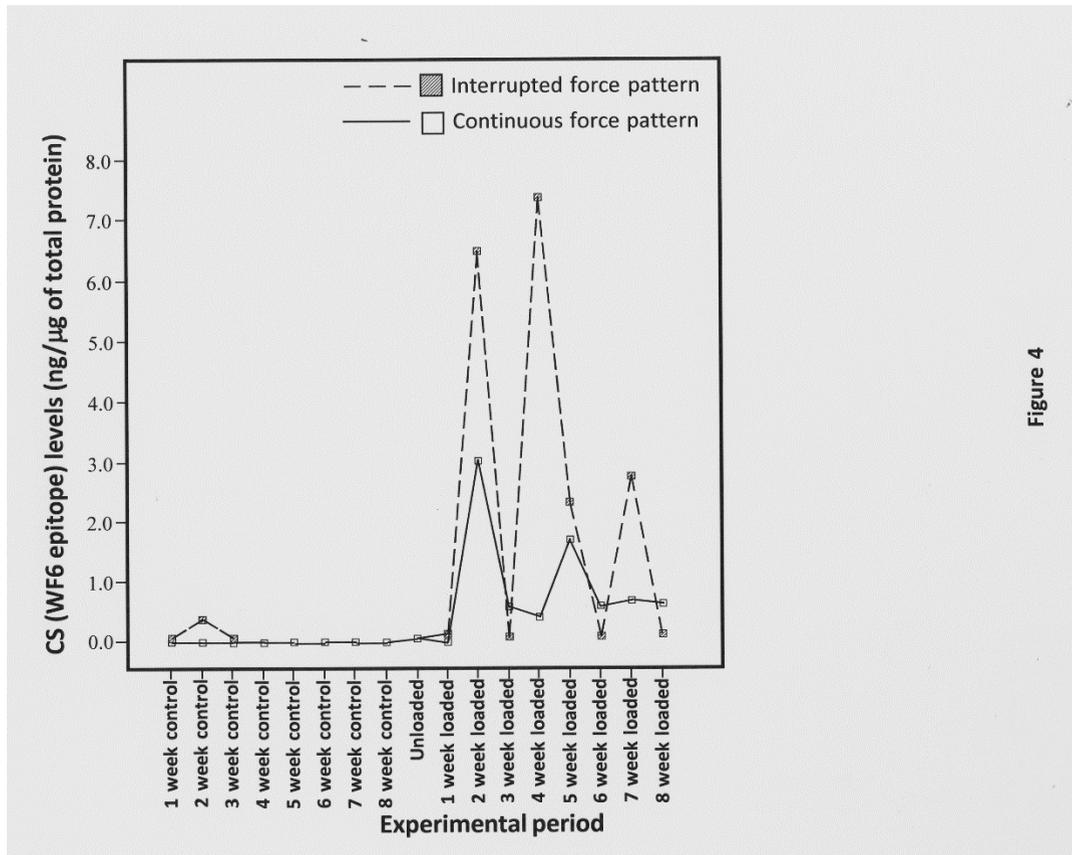
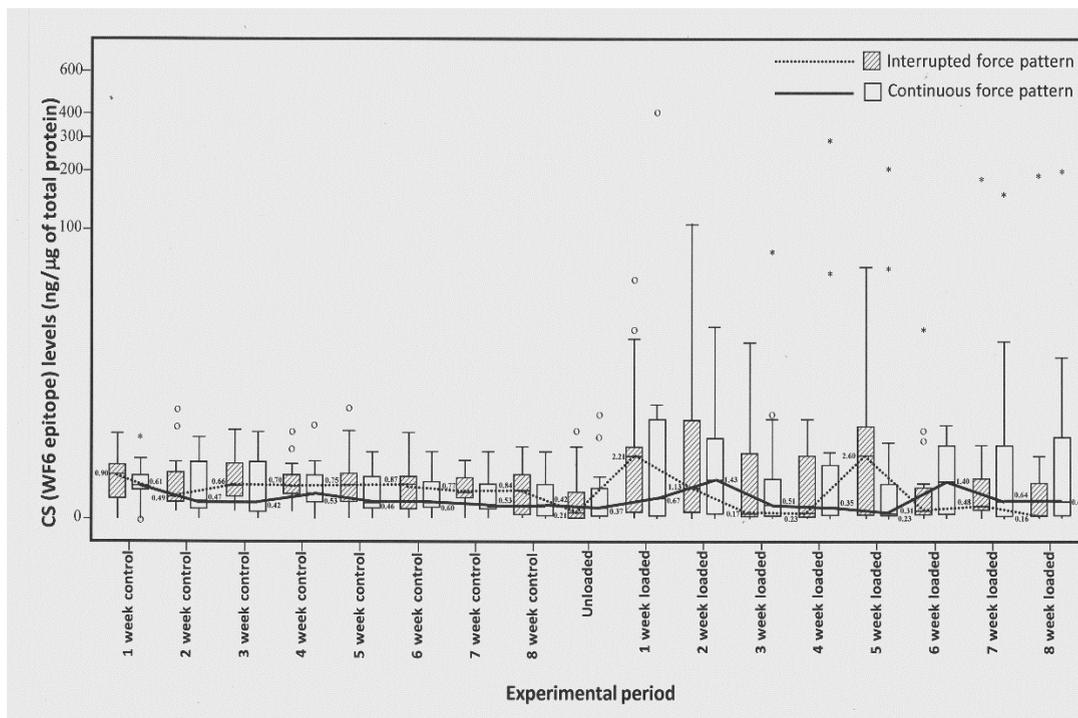


Figure 4

**Fig. 4** Profile graph of the chondroitin sulphate (CS; WF6 epitope) levels around the right (interrupted force pattern) and the left (continuous force pattern) mandibular canines, from a subject, during the 8-week control, the unloaded and the 8-week loaded periods

The medians of CS levels around the right (interrupted force pattern) and the left (continuous force pattern) experimental mandibular canines during each 1 week period (the 8-week control, the unloaded and the 8-week loaded periods) are shown in Fig. 5. During the 8-week control period (control data) and during the unloaded period (baseline data), the medians of CS levels around the right (interrupted force pattern) mandibular canines were 0.90, 0.49, 0.66, 0.70, 0.75, 0.87, 0.77, 0.84 and 0.21 ng/μg

of total protein, respectively, and the medians of CS levels around the left (continuous force pattern) mandibular canines were 0.61, 0.47, 0.42, 0.53, 0.46, 0.60, 0.53, 0.42 and 0.37 ng/μg of total protein, respectively. There was no significant differences in the median CS levels between the control and the unloaded periods, and between the right and the left mandibular canines.



**Fig. 5** Boxplot graphs of the chondroitin sulphate (CS; WF6 epitope) levels around the right (interrupted force pattern) and left (continuous force pattern) experimental mandibular canines during each 1 week period during the 8-week control, the unloaded, and the 8-week loaded periods. Note the cyclical pattern of a broken line connecting the median CS levels in gingival crevicular fluid (GCF) samples of right mandibular canines, and of a solid line connecting the median CS levels in GCF samples of left mandibular canines. The small asterisks represent the extreme values.

During the 8-week loaded period (experimental data), the medians of CS levels around the right (interrupted force pattern) mandibular canines were 2.21, 1.13, 0.17, 0.23, 2.60, 0.31, 0.48, and 0.16 ng/µg of total protein, respectively, and the medians of CS levels around the left (continuous force pattern) mandibular canines were 0.67, 1.43, 0.51, 0.35, 0.23, 1.40, 0.64, and 0.63 ng/µg of total protein, respectively. There was no significant differences in the medians of CS levels between baseline and experimental data, and the medians of CS levels in GCF of the right and those of the left experimental mandibular canines during each one-week loaded period.

**No difference in the mean rates of space closure and in the medians of VAS scores of the patients' pain and discomfort between two different force patterns**

The mean rate of space closure by interrupted force pattern was  $0.70 \pm 0.55$  mm/month, and that by continuous force pattern was  $0.86 \pm 0.37$  mm/month (Table 1). Comparisons of mean rates showed statistically insignificant differences. At the end of first week, the medians of VAS scores of the patients' pain and discomfort resulting from interrupted (5.33) and from continuous (4.89) force patterns were not significantly different, and at the end of fifth week, the medians of VAS scores of the patients' pain and discomfort from interrupted (4.44) and from continuous (5.11) force patterns were not significantly different.

**Table 1** The rates of space closure [in millimeters (mm)/month] are shown as the minimum, maximum, mean, and standard deviation between two different force patterns ( $n = 15$ )

Force Patterns	Rates of space closure (mm/month)			
	Minimum	Maximum	Mean	Standard Deviation
Interrupted	0.25	1.67	0.70	0.55
Continuous	0.25	2.17	0.86	0.37

## DISCUSSION

Previously, we applied our patented WF6 monoclonal antibody, raised against WF6 catabolic epitope of CS<sup>13</sup> to monitor changes of CS levels in peri-miniscrew implant crevicular fluid (PMICF) during orthodontic loading<sup>11</sup>, in GCF of intruded maxillary molars,<sup>14</sup> and in GCF of moved maxillary canines under two different force magnitudes.<sup>9</sup> In this present study, we evaluated the effects of either interrupted (generated by elastomeric chains) or continuous (generated by Nickel-Titanium closed coil spring) orthodontic force pattern by monitoring changes in CS levels in GCF of orthodontically moved mandibular canines.

To evaluate the effects of either interrupted or continuous force during orthodontic loading, some investigations selected various inflammatory mediators in GCF such as interleukin-1 $\beta$ , interleukin-8 and prostaglandin E<sub>2</sub> as biomarkers.<sup>7,15</sup> Leethanakul and colleagues reported a significant greater elevation of interleukin-1 $\beta$  and interleukin-8 levels at 24 hours after the first activation, compared with the control sites. Our present study used the levels of CS which was a kind of tissue break down products, detected in GCF, as biomarker for assessing alveolar bone remodeling and periodontal response to orthodontic loading. In our study, the medians of CS levels were elevated 1-2 weeks after force application with either interrupted or continuous force pattern, and then it gradually decreased.<sup>7</sup> For both orthodontic force patterns during the loaded period, CS levels showed cyclical changes at the 3-4 week intervals, and this was consistent with bone turnover rate. These changes comprised many peaks of high CS level during the loaded period. Compared to the cyclical changes in CS levels during the loaded period, the pattern of CS levels during the control period remained very low, and this was similar to that detected in periodontally healthy sites,<sup>16</sup> and that in the control group as reported by Insee *et al.*<sup>9</sup>

Leethanakul and colleagues reported that IL-1 $\beta$  and IL-8 levels caused by a continuous force pattern were significantly higher than those caused by an interrupted force pattern during all experimental periods, and suggested that continuous force pattern (generated by Nickel-Titanium closed coil spring) had a greater effect on cellular activity than did interrupted force pattern (generated by elastomeric chains).<sup>7</sup> However, our present study showed that the effects of both orthodontic force patterns on CS levels were not significantly different. The difference between those two studies may be due to different experimental sites (maxillary canines versus mandibular canines) and different initial force magnitude (170 cN versus 120 g). While the tooth is moved by orthodontic force, deflection of alveolar bone and remodeling of periodontal

tissues occur. Alveolar bone deflection can be just started in response to an initial orthodontic force and then osteocytes behave as mechanoreceptors. Stress produced in alveolar bone by orthodontic force can immediately generate electrical effects and may cause bone remodeling.<sup>8</sup> The initial orthodontic force magnitude, both continuous and interrupted force pattern, may produce rapid changes in metabolic activity of alveolar bone after being applied to a tooth. Investigations relating to the response of alveolar bone remodeling to various initial orthodontic force magnitude should be further carried out.

Our results showed that mean rate of space closure by continuous force pattern was insignificantly different from that by interrupted force pattern. These results agreed with those reported by Lee and colleagues.<sup>15</sup> Our results also agreed with those reported by Nightingale and Jones<sup>17</sup>, although they investigated the efficiency of continuous and interrupted force patterns during maxillary anterior contraction. Many previous studies reported that continuous force pattern produced significantly higher rates of canine movement than did interrupted force pattern<sup>1,4,5,7</sup>; however, those previous studies used higher initial force magnitudes (150-200 g for Nickel-Titanium closed coil springs and 170-450 g for elastomeric chains) than that used in our present study (120 g initial force magnitude). At the beginning of the loaded period, our subjects reported similar pain and discomfort for both right (interrupted) and left (continuous) mandibular canines because of similar 120 g initial force application. Although the remaining interrupted force was gradually decreased after force application as shown in Fig. 2 (for example 60.0 g at 1-week loaded and 62.5 g at 5-week loaded), our subjects still reported similar VAS scores of pain and discomfort for both force patterns. Our results agreed with that of Samuels and colleagues.<sup>4</sup> however, they used 150 g continuous force pattern and initial 400-450 g interrupted force pattern, and VAS score evaluation of pain and discomfort was not implemented.

In our present study, the median of CS levels was highest at 1-week and 5-week loaded periods after force application with interrupted force pattern, and at 2-week and 6-week loaded periods after force application with continuous force pattern. These results showed cyclical pattern of elevated CS levels (or alveolar bone remodeling) with 3-4 week interval during the loaded periods, in contrast to the unloaded or control periods which showed non-cyclical pattern. Bone remodeling process involves osteoclastic bone resorption and osteoblastic bone formation. Osteoclasts act as major resorbing cells in bone remodeling process, and have a limited life span of 12.5 days.<sup>18,19</sup> Therefore, the peak levels of CS

1 to 2 weeks after force activation found in this present study are consistent with average life span of human osteoclasts.

It should be noted that all experimental data during the 8-week loaded period have been pooled. So, during the 8-week loaded period (experimental data), the medians of CS were significantly greater than those during the unloaded period (baseline data). In the contrary, if all experimental data during the 8-week loaded period have been divided into 8 one-week periods, and then compared. The resulted showed that there were no significant differences in the medians of CS levels between baseline and experimental data. The reason is that the number of sample in each group of one-week period has been decreased, and this resulted in a statistically non-significant difference.

Both interrupted and continuous force patterns, with 120 g initial force magnitude, are within the optimum range of orthodontic force for mandibular canine movement, so we propose that the 'initial' force magnitude, rather than force magnitude alone, plays an important role in triggering the biological processes relating to alveolar bone remodeling because two different force patterns with same 120 g initial force magnitude cause no difference in biochemically assessed bone remodeling activity, the same patients' pain and discomfort, and the same rate of tooth movement during mandibular canine movement. Suggestions for further studies are that other biomarkers which are closely related to osteoclastic activity or to root resorption process should also be simultaneously monitored.

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