Original Article

Interleukin 1β Levels around Microscrew Implants during Orthodontic Tooth Movement

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ABSTRACT

Objective: To determine whether interleukin 1β (IL-1β) levels are elevated around microscrew implants that are used as anchorage for tooth movement.

Materials and Methods: Ten young adults, aged 16.3 ± 2.5 years and with all four premolars extracted , comprised the study group. Twenty maxillary microscrew implants were placed bilaterally in the alveolar bone between the maxillary second premolars and first molars as anchorage units for distal movement of the maxillary canines. The maxillary canines served as the treatment group, and the microscrew implants were designated as the implant group. The mandibular canines were used as controls. Peri-microscrew implant crevicular fluid (MICF) and gingival crevicular fluid (GCF) were collected at the beginning of tooth movement (2 weeks after implant placement); at 24, 48, and 168 hours later; and on days 14 and 21. An automated enzyme immunoassay was used to measure 1L-1β in the MICF and the GCF.

Results: The mean 1L-1β level in the treatment group was significantly elevated at 24 hours ($P = .003 < .05$) and 48 hours ($P = .003 < .05$), whereas the levels in the control and implant groups did not change significantly during the experimental period. Also, the mean 1L-1β level of the treatment group was significantly higher that in both the control and implant groups at 24 and 48 hours.

Conclusions: The microscrew implants did not demonstrate increased 1L-1β levels during tooth movement. This supports the concept that microscrew implants might be useful as absolute anchorage devices.

KEY WORDS: Microscrew; Interleukin-1β; Gingival crevicular fluid

INTRODUCTION

Orthodontic forces induce chemical and physical responses in the periodontal tissues. At the beginning of orthodontic tooth movement, the mechanical stimulus causes an acute inflammatory reaction within the periodontal tissues, which in turn may trigger the biologic processes that result in bone resorption to accommodate movement of the tooth.[1–3] The mechanism of bone resorption may induce the release of inflammatory mediators, such as interleukin-1, at the biomolecular level. Interleukin-1 exists in two forms: alpha (IL-1α) and beta (IL-1β). Both induce bone resorption, but IL-1β seems to be a more potent inducer of resorption and inhibits bone formation.[3,4]

The treatment of Class II malocclusion often requires intrusion and retraction of the anterior segment, which, in turn, usually necessitates mechanical reinforcement of posterior anchorage.[5] Transpalatal bars or Nance appliances, such as an intraoral anchorage device, can reduce the need for Class II elastics but can also cause mesial movement of the mandibular first molars and protrusion of the incisors. On the other hand, extraoral anchorage in the form of a headgear is often rejected by adult patients for social and professional reasons. In addition, if a headgear is worn for 14 hours a day, some loss of anchorage and mesial movement of maxillary molars are also observed.[6,7]

The use of dental implants,[8] miniplates,[9] miniscrews,[10] and microscrews[11,12] has expanded, since these devices provide absolute anchorage, they are small enough to place in any area of the alveolar bone, and they are easy to place and remove. Moreover,

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Deguchi et al[13] reported that orthodontic force application should be applied almost immediately after placement, in contrast to dental implants. Park and Kwon[14] also demonstrated the efficacy of microscrew implants for anchorage control during retraction of maxillary anterior teeth, for vertical control of mandibular posterior teeth, and for vertical control of the facial profile in three patients. However, the effect of microscrew implants at the biomolecular level has not been evaluated.

Meffert[15] indicated that when implant failure occurs, it is clinically accompanied by increased probing depth, patient reports of pain, and/or radiographic bone loss. This process has been named peri-implantitis. Kao et al[16] reported that IL-1β could be identified in the implant crevicular fluid (ICF) and it should be used as a marker for monitoring the health status of dental implants. They also observed that the levels of IL-1β were significantly higher in patients with failing implants versus those with healthy implants.

The purpose of this study was to evaluate the IL-1β levels in healthy peri-microscrew implant crevicular fluid (MICF) and compare these with the IL-1β levels in healthy gingival crevicular fluid (GCF) around natural teeth during 3 weeks of distal canine movement.

MATERIALS AND METHODS

Ten adult orthodontic patients with Class II malocclusion, aged 16.3 ± 2.5 years comprised this study. Informed consent was obtained from all patients.

These patients met the following criteria:

- All four first premolars had been extracted.
- General health was good, with a healthy periodontium, no radiographic evidence of bone loss, no gingival inflammation, and a probing depth of 3 mm or less at all teeth.
- Patients had not used antibiotics or anti-inflammatory drugs in the month preceding the study.
- The women were not pregnant.

Twenty healthy microscrew implants with no accompanying gingival inflammation and no peri-implant pockets greater than 3 mm, which were accepted as healthy by the criteria of Aboyoussef et al,[17] were considered in this study.

A complete fixed preadjusted edgewise appliance with 0.018-inch slots was attached, and a 0.014-inch nickel-titanium (NiTi) archwire was placed for initial leveling. After the maxillary anterior teeth were aligned, a 0.016- × 0.022-inch blue Elgiloy archwire with molar toe-ins and tip-back bends was placed, and the maxillary second premolars and first molars were ligated together before beginning distal movement of the canines.

Twenty microscrew implants (8 mm long, 1.6 mm diameter, Neo Anchor Plus, Myungsung, Seoul, Korea) were placed bilaterally into the interradicular bone between the maxillary second premolars and first molars in 10 patients, as described in previous reports.[18,19] Two weeks after placement, distal movement of the maxillary canines was begun with a 120-g force delivered by a NiTi closed-coil spring between the microscrew implants and canines.

The mandibular (antagonistic) canines were chosen as controls because of the similarity of anatomy. Archwires and brackets were placed in the mandibular arch in the same way as in the maxilla, but the mandibular canines were not moved distally.

Collection of Gingival Crevicular Fluid and Microscrew Implant Crevicular Fluid Samples

GCF samples were obtained from the maxillary canines (treatment group) and both of the mandibular canine teeth (controls). MICF samples were also collected. All samples were obtained over 3 weeks according to following schedule:

T1 (baseline): 1 hour after activation of the closed-coil springs
T2: 24 hours after activation
T3: 48 hours after activation
T4: 168 hours after activation
T5: 14th day after activation
T6: 21st day after activation

GCF and MICF sampling were performed in the clinic at approximately 20°C and 40% relative humidity between 9:00 and 10:00 AM. Four strips of filter paper were used to sample the GCF around the maxillary canines and were put in one Eppendorf tube. Another four filter papers were used to collect MICF and placed in another Eppendorf tube. Similarly, four filter papers were used for the control teeth. All filter papers were autoclaved and weighed on a digital scale (Metler AT-210, Mettler-Toledo Inc, Columbus, OH) before use.

After activation of the closed-coil springs, the peri-implant and marginal gingival areas of the canine teeth were isolated with cotton rolls and dried with a gentle stream of air. Bilaterally, MICF samples were collected from the mesiobuccal aspects of the microscrew implants. Meanwhile, GCF samples were also obtained bilaterally at both distobuccal sites of the maxillary canines. Two filter papers (0.2 cm) for GCF and another two filter papers for MICF were inserted into the base of the pocket in both sides until slight resistance was felt. These papers were left in place for 3 minutes. Samples containing blood were discarded. Acceptable filter papers were put in the Eppendorf tubes and

Table 1. Within-Group Comparisons of Baseline IL-1β Levels (pg/μL) and IL-1β at Different Time Points[a]

Group[b]	T1 (baseline)	T2 (24 h)	*P* T1 vs T2	T3 (48 h)	*P* T1 vs T3	T4 (168 h)	*P* T1 vs T4	T5 (14 d)	*P* T1 vs T5	T6 (21 d)	*P* T1 vs T6
Treatment	17.8 ± 4.3	37.8 ± 6.7	.003*	28.6 ± 6.9	.003*	21.2 ± 4.7	.007	20.6 ± 4.3	.008	19.7 ± 2.6	.065
Control	18.3 ± 3.4	19.6 ± 2.6	.0192	20.6 ± 1.8	.004	19.8 ± 2.6	.053	20.0 ± 3.0	.017	20.2 ± 2.3	.007
Implant	21.1 ± 3.0	22.0 ± 2.5	.014	22.3 ± 3.0	.116	21.6 ± 2.8	.275	20.7 ± 2.7	.305	21.5 ± 3.2	.667

[a] Values are mean ± standard deviation; * $P < .05$.
[b] For each group, n = 10.

weighed again to determine the volume of fluid collected.

Sterilized saline solution (250 μL) was added to the Eppendorf tubes and samples were centrifuged for 1 minute. All cytokines were recovered from the paper strips by 5 minutes of centrifugal elution. The papers were then removed and the solutions were stored at −70°C until the immunoassay was performed. GCF samples of mandibular canines in the control group were also performed on the same schedule and in the same manner.

Interleukin 1β Assay

A commercial IL-1β enzyme-linked immunosorbent assay (ELISA) kit (BioSource, Camarilo, Calif) was used to determine the IL-1β levels. Fifty microliters of each sample and standards were applied to each well, which had been precoated with anti-human IL-1β antibodies, in duplicate. Then 100 μL of biotinylated anti–IL-1β were added; the side of the plate was tapped gently to mix. The plate was covered with a plate cover and incubated for 2 hours at room temperature (20°C to 25°C). After washing four times with a wash buffer, 100 μL streptavidin—horseradish peroxidase working solution was added to each well, except for the chromogen blanks. Then the plate was covered with the plate cover and incubated for 30 minutes at room temperature. One hundred microliters of stabilized chromogen were added to each well and allowed to react for 25 minutes at room temperature and in the dark. The liquid in the wells began to turn blue. Then 100 μL of stop solution was added to each well, the solution in the wells changed from blue to yellow, and the optical densities (OD) were read at 450 nm on an ELISA reader (EL 312e, Biotek Instruments, Winooski, Vt) within 2 hours.

Table 2. Within-Group Comparisons of IL-1β (pg/μL) Levels at T2 with IL-1β Levels at T3, T4, T5, and T6[a]

Group[b]	*P* T2 vs T3	T2 vs T4	T2 vs T5	T2 vs T6
Treatment	.003*	.003*	.003*	.003*
Control	.163	.858	.633	.304
Implant	.751	.673	.056	.378

[a] Values are mean ± standard deviation; * $P < .05$.
[b] For each group, n = 10.

Statistical Analysis

The data were analyzed with SPSS software (SPSS, Chicago, Ill). Within-group differences in IL-1β levels between T1 and T6 were evaluated by the Wilcoxon signed-rank test. The differences between the IL-1β levels of the groups at baseline and at T2, T3, T4, T5, and T6 were determined by the Mann-Whitney *U*-test. Results were considered statistically significant at $P < .05$.

RESULTS

All 10 subjects completed the study, and MICF was obtained from 20 microimplants, whereas GCF was collected from 10 maxillary canines as the treatment group and 10 mandibular canines as the control group.

Intragroup Differences

After orthodontic activation (T1), IL-1β values of GCF/MICF were 17.8 pg/μL in the treatment group, 18.3 pg/μL in the control group, and 21.1 pg/μL in the implant group (Table 1). IL-1β values of GCF in the treatment group had increased significantly, to 37.8 pg/μL at T2, compared with baseline values ($P = .003$). At T3, the IL-1β values (28.6 pg/μL) of the treatment group were, again, significantly higher than at baseline ($P = .003$). However, no statistical differences were observed between baseline and T4 ($P = .007$), baseline and T5 ($P = .008$), and baseline and T6 ($P = .065$) in the treatment group. On the other hand, IL-1β values in the implant and control groups did not change significantly at any time during the experiment (Table 1).

In the treatment group, IL-1β values of GCF decreased significantly versus the T2 value (Table 2): readings were 28.6 pg/μL at T3 ($P = .003$), 21.2 pg/μL at T4 ($P = .003$), 20.6 pg/μL at T5 ($P = .003$), and 19.7 pg/μL at T6 ($P = .003$). These findings were also statistically different. In contrast, none of the IL-1β values in the implant group or control group between T2 and T3, T2 and T4, T2 and T5, and T2 and T6 were statistically significantly different (Table 2).

Table 3. Within-Group Comparisons of IL-1β (pg/μL) Levels at T3 with IL-1β Levels at T4, T5, and T6[a]

	P		
Group[b]	T3 vs T4	T3 vs T5	T3 vs T6
Treatment	.003*	.003*	.003*
Control	.322	.472	.439
Implant	.223	.026	.176

[a] Values are mean ± standard deviation; * $P < .05$.
[b] For each group, n = 10.

In the treatment group, the level of IL-1β decreased by 7.2 pg/μL ($P = .003$) between T3 and T4, by 8 pg/μL ($P = .003$) between T3 and T5, and by 8.9 pg/μL ($P = .003$) between T3 and T6. These decreases were also significantly different, whereas IL-1β levels in the implant and control groups did not change statistically significantly during these periods (Table 3). In all three groups, significant differences in IL-1β levels were not observed between T4 and T5, between T4 and T6, and between T5 and T6 (Table 4).

Intergroup Differences

At T2, the mean level of IL-1β in GCF of the treatment group was 37.8 pg/μL, whereas it was 19.6 pg/μL in the control group (significantly different versus treatment group, $P = .000$) and 22.0 pg/μL in the implant group (significantly different versus implant group ($P = .000$) (Table 5). The mean 1L-1β level in the treatment group was 28.6 pg/μL at T3, which was statistically significantly different from both the control group ($P = .002$) and the implant group ($P = .012$). No significant differences were found between the treatment and control groups at T4, T5, and T6. In addition, no significant changes were present between the treatment and implant groups at T4, T5, and T6. Finally, no significant differences in IL-1β levels were observed between the control and implant groups at any of the time points (Table 5; Figure 1).

DISCUSSION

Orthodontic tooth movement is a complex orchestration of piezoelectric responses, prostaglandin production, and the action of various extracellular or intracellular biochemical factors.[20,21] Meanwhile, some cytokines are released into the gingival crevicular fluid, and those cytokines can be evaluated with ELISA.[1,16] Some studies have previously shown that IL-1β is present in the peri-implant crevicular fluid. It is also reported that IL-1β levels may provide a means of monitoring the health status of dental implants.[16,22–24] On the other hand, IL-1β produces a wide range of biologic effects, including prostaglandin E_2 (PGE_2) synthesis, stimulation of collagenase, and inhibition of bone formation.[25,26]

Table 4. Within-Group Comparisons of IL-1β (pg/μL) Levels at T4 with IL-1β Levels at T5 and T6[a]

	P		
Group[b]	T4 vs T5	T4 vs T6	T5 vs T6
Treatment	.427	.121	.169
Control	.889	.471	.753
Implant	.067	.957	.102

[a] Values are mean ± standard deviation; * $P < .05$.
[b] For each group, n = 10.

Table 5. Between-Group Comparisons of IL-1β (pg/μL) Levels at All Time Points[a]

	P		
Time	(Treatment vs control)	(Treatment vs implant)	(Control vs implant)
0 h	.766	.055	.043
24 h	.000*	.000*	.034
48 h	.002*	.012*	.103
168 h	.427	.973	.167
14 d	.528	.74	.921
21 d	.596	.194	.485

[a] Values are mean ± standard deviation; * $P < .05$.

Aboyoussef et al[17] examined ICF samples for the presence and levels of PGE_2. They reported that the concentration of PGE_2 in the ICF of healthy sites did not differ significantly from that at the ICF of diseased sites. However, the levels of IL-1β were significantly higher in patients with failing implants than in patients with healthy implants.[16,27] That is why 1L-1β was the preferred cytokine for this study.

Microscrew implants have recently been used to enhance orthodontic anchorage without patient compliance. They have many advantages, such as ease of placement and removal, small size, and low cost.[11–13]

In our study, IL-1β levels in peri-microscrew crevicular fluid were determined in a manner similar to that used for peri-implant crevicular fluid in previous studies.[16,23,24] IL-1β levels in the treatment group increased significantly over baseline measurements after 24 to 48 hours of orthodontic force application. By 168 hours, 14 days, and 21 days, IL-1β levels had subsided to nearly baseline levels. The decline in the orthodontic force at these time points most likely accounts for this. These findings coincide with those of Grieve et al[3] and our previous studies[2] (studied with PGE_2 synergistic with IL-1β). However, the changes in IL-1β levels of peri-microscrew crevicular fluid were not statistically significant during the 3-week period, whereas the IL-1β values of GCF in the treatment group did change significantly.

Several studies have shown that the levels of IL-1β in the peri-implant crevicular fluid are significantly higher in patients with failing implants than in those

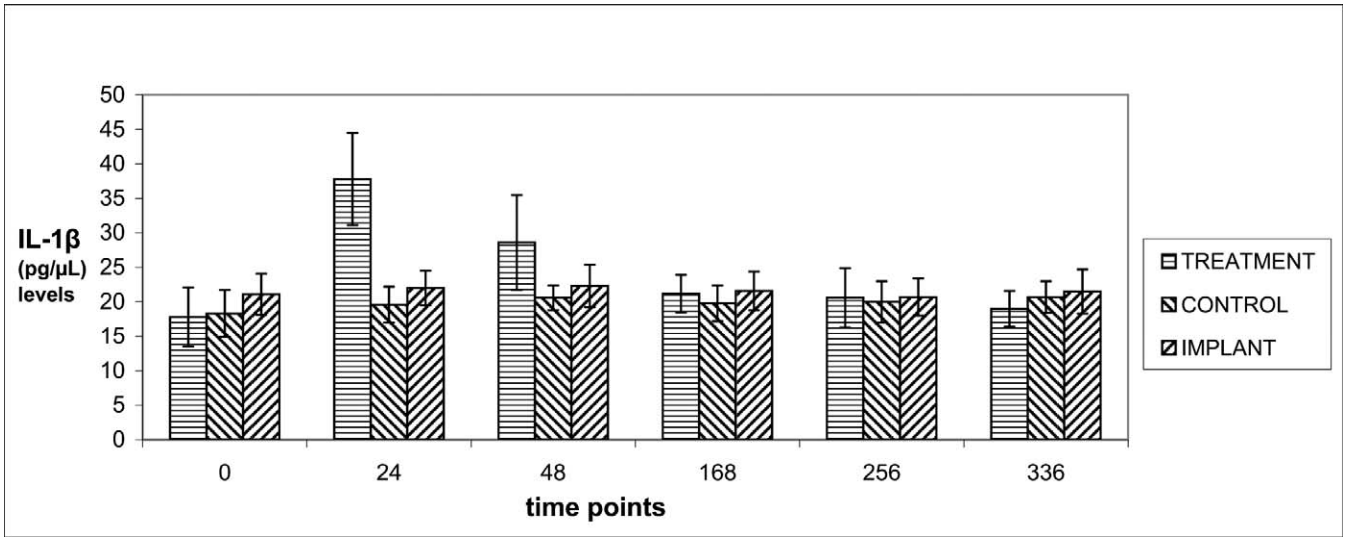

Figure 1. IL-1β (pg/μL) levels in treatment, control, and implant groups at all five time points (0 hours, 24 hours, 48 hours, 14 days, and 21 days).

with healthy implants.[16,23,24] The fact that there were no statistical differences in IL-1β levels of the peri-microscrew crevicular fluid during the 3 weeks of this study may be a reflection of the fact that the microscrews used in the study were healthy. The intergroup comparisons showed no statistically significant differences in IL-1β levels between the control and implant groups. This also indicated that mechanical stress on healthy microscrews may not affect the levels of IL-1β in peri-microscrew crevicular fluid. However, the results of this pilot study need to be confirmed with longer study periods and sampling of different cytokines.

CONCLUSION

- The implants did not demonstrate increased 1L-1β levels during tooth movement. This supports the concept that microscrew implants might be useful as absolute anchorage devices.

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