Original Article

Impact of orthodontic retainers on periodontal health status assessed by biomarkers in gingival crevicular fluid

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ABSTRACT

Objective: To evaluate whether biomarkers of inflammation and periodontal remodeling are differentially expressed in the gingival crevicular fluid (GCF) of patients wearing different types of orthodontic retainers.

Materials and Methods: Thirty-one adult subjects (17 men and 14 women with an age range of 20 to 35 years) were allocated to three different groups. Group 1 consisted of 10 patients wearing fixed retainers, group 2 included 11 patients using lower removable retainers, and group 3 comprised 10 patients without retainers (control). Periodontal health assessment and GCF collection were carried out at two sites per subject: the lingual side of a central lower incisor and the lingual side of a lower second premolar. Aliquots from diluted GCF were screened for the presence of biomarkers using a microarray technique.

Results: Group 1 patients exhibited a higher percentage of sites with visible plaque in the incisor region than the other groups ($P = .03$); no differences were noted in gingival bleeding and probing depths. The median concentrations (pg/mL) of interferon-gamma and interleukin-10 were significantly higher in the premolar sites of patients in group 2 ($P = .01$ and $P = .04$, respectively), whereas the concentration of matrix metalloproteinase-9 was significantly higher at the incisors of patients wearing fixed retainers ($P = .02$). A significant difference between the two sites was seen only in group 2.

Conclusions: The presence of different orthodontic retainers may promote specific alterations in the GCF composition. With retention periods potentially becoming longer, this finding may be of clinical significance. (*Angle Orthod.* 2011;81:1083–1089.)

KEY WORDS: Gingival crevicular fluid; Orthodontic retainers; Periodontal disease; Biomarkers; Protein microarrays

INTRODUCTION

Alignment instability is one of the major pitfalls of orthodontic treatment. As a result, orthodontists tend to recommend long-term use of retainers for enhanced stability, which may require years or even decades of retainer wear.[1] A recent systematic review[2] stated that there are insufficient research data on which to base clinical decisions regarding retention. Accordingly, the potential increase in duration of retention makes it important to evaluate the outcome of long-term retention on surrounding tissues, with a particular emphasis on periodontal disease.

Periodontal destruction is induced by the deleterious effects of inflammatory mediators that appear as a result of bacterial plaque buildup around the tooth.[3] Interestingly, the majority of published studies[3–6] focused on bacterial plaque accumulation and clinical

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Accepted: April 2011. Submitted: January 2011.
Published Online: June 9, 2011
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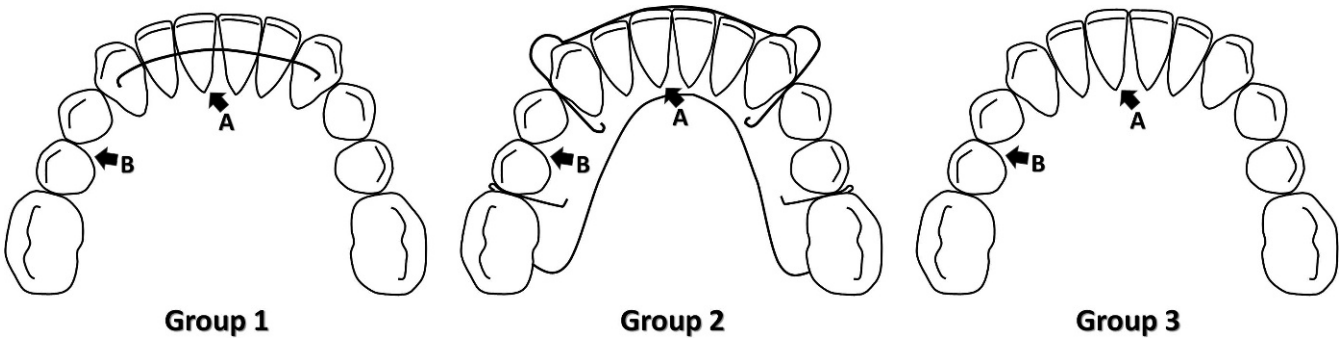

Figure 1. Collection sites for GCF in group 1 (fixed retainer), group 2 (removable retainer), and group 3 (no retainer). Site A indicates gingival sulcus at the lingual side of the mandibular central incisor; site B, gingival sulcus at the lingual side of the mandibular second premolar.

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periodontal health assessment in the presence of retainers. Clinical methods to diagnose periodontal disease are very cost-effective, but they do not distinguish between disease-active and disease-inactive periodontal sites. On the other hand, biochemical analysis of the gingival crevicular fluid (GCF) offers a noninvasive means to assess the host response in periodontal disease.[7] GCF is a complex biological fluid that contains a mixture of serum-derived and inflammatory proteins.[8] The constituents of GCF arise from a variety of sources, including microbial plaque, host cells, and serum-derived factors; thus, a number of biomarkers are available to examine in the GCF.[7,8]

Matrix metalloproteinase-9 (MMP-9) is derived predominantly from monocytes and macrophages, and its levels in GCF are higher in patients with periodontitis; therefore, MMP-9 may serve as a biomarker of periodontal disease and aid in early detection of periodontitis.[9] An intermediate mechanism that lies between bacterial stimulation and tissue remodeling is the production of cytokines. Interferon-gamma (IFN-γ) is a proinflammatory cytokine that increases in experimental gingivitis and periodontitis.[8] On the other hand, interleukin-10 (IL-10) is an anti-inflammatory cytokine that inhibits the release of proinflammatory analytes.[10] All these cytokines play key roles in immune and inflammatory responses, and infection outcome may be attributable to the balance in the relative ratios among all of them.

The specific aim of this project was to evaluate whether biomarkers of inflammation and periodontal remodeling, such as MMP-9, IFN-γ, and IL-10, are differentially expressed in the GCF of patients who have worn retainers for an extended period of time. The authors hope to provide clinicians with a better understanding of the effect of long-term retainer use on periodontal tissues, which will enable them to make better decisions for their patients.

MATERIALS AND METHODS

Thirty-one individuals (17 men and 14 women with an age range of 20 to 35 years) were included in the study. To be included, subjects needed to have completed full fixed orthodontic treatment at least 4 years before the initiation of the study, to be free of systematic disease, and to have received no periodontal treatment in the last 6 months. Smokers and drug users were excluded from the study. Written informed consent was obtained from the participants, and ethical approval was granted from the Health Research Ethics Board at the University of Manitoba (reference number H2009:15).

Subjects were allocated into three groups. Group 1 consisted of 10 patients who wore retainers bonded to the lingual surfaces of both lower canines. The fixed mandibular retainers in this group had been placed upon the completion of treatment and had been constructed of nonbraided 0.028-inch round stainless steel wire. Group 2 consisted of 11 patients who used removable lower Hawley-type retainers regularly on a daily basis. Ten postorthodontic patients wearing no retainers were placed in the control group (group 3). The mean period of retention was 5.6 years (range, 4 to 10 years).

Clinical periodontal health assessment and GCF collection were carried out at two sites per subject: the lingual side of a lower central incisor and the lingual side of a lower second premolar (Figure 1). It was deemed necessary to choose those teeth because different biological processes might be involved at sites directly influenced by the fixed retainer, such as the gingival sulcus on the lingual side of the lower incisors.

Probing depth (PD), measured with a straight periodontal probe, was recorded as the distance in millimeters from the gingival margin to the most apical part of the sulcus.[11] Presence or absence of bleeding

Table 1. Demographic Data, Periodontal Parameters,[a] and Gingival Crevicular Fluid (GCF) Volume (mean ± SD) of the Studied Subjects

	Group 1 (n = 10)	Group 2 (n = 11)	Group 3 (n = 10)
Gender (M:F)	3:7	9:2	5:5
Age (y)	28 ± 4.9	24 ± 3.6	26.9 ± 4.22
PD, incisors (mm)	1.85 ± 0.81	1.68 ± 0.46	1.7 ± 0.63
PD, premolars (mm)	2.15 ± 0.94	2.04 ± 0.56	2.05 ± 0.59
PA, incisors (%)	60 ± 51.6*	18.18 ± 40.45	10 ± 31.6
PA, premolars (%)	10 ± 31.6	9.09 ± 30.1	0 ± 0
BOP, incisors (%)	30 ± 48.3	0 ± 0	20 ± 42.16
BOP, premolars (%)	20 ± 42.16	18.18 ± 40.45	20 ± 42.16
GCF volume, incisors (μL)	0.24 ± 0.3	0.14 ± 0.04	0.15 ± 0.06
GCF volume, premolars (μL)	0.15 ± 0.07	0.22 ± 0.130	0.15 ± 0.06

[a] PD indicates probing depth; PA, plaque accumulation; and BOP, bleeding on probing.
* Statistically significant difference ($P \leq .05$).

on probing (BOP) and plaque accumulation (PA) were recorded in a dichotomous manner (0 or 1): If bleeding occurred within 15 seconds after retrieval of the probe, the site was recorded as BOP-positive, and the presence of visible plaque after running the probe along the gingival margin was recorded as positive.[11,12]

Before GCF collection, supragingival plaque was removed with a plastic scaler; then, each site was gently dried for 10 seconds with compressed air and isolated from saliva with a cotton roll. GCF collection at all sites was performed using paper strips (Periopaper, Oraflow Inc, Plainview, NY). Two strips were inserted for 30 seconds into the gingival sulcus of each selected tooth, with 30 seconds between samplings. The volume of GCF was determined by positioning the strips between the upper and the lower counterparts of the precalibrated Periotron 8000 (Periotron 8000, Oraflow Inc). The paper strips were then placed in a single labeled Eppendorf tube containing 80 μL phosphate-buffered saline (Invitrogen, Camarillo, Calif), sealed, and immediately sent to the laboratory. Supernatant samples from all patients were stored at −80°C for subsequent biomarker analysis.

Biomarker Analysis

To ensure that the samples included enough proteins to be quantified, 5 μL of diluted GCF from all sites were submitted to a general protein quantification assay, as previously described by Desjardins et al.[13] Subsequently, sample aliquots from diluted GCF from all groups' sites were screened for the presence of specific proteins, including IL-10, IFN-γ, and MMP-9, with a customized Quantibody Array (RayBiotech, Norcross, Ga). One standard glass slide was spotted with 16 wells of identical biomarker antibody arrays. Each antibody, together with the positive and negative control, was arrayed in quadruplicate.[14,15] Then, after 30 minutes' incubation with sample diluent, the glass chips were washed, and each well was overlaid with 50 μL of diluted GCF. After overnight incubation at 4°C and extensive washing, the detector antibody was added for 1 hour and then washed away; Alexa fluor 555-conjugated streptavidin was then added for 2 hours at room temperature. The signals (Cy3 wavelengths: 555 nm excitation, 655 nm emission) were scanned and extracted with a Genepix 4000B laser scanner (Axon Instruments, Foster City, Calif). Both the total amounts and concentrations of biomarkers were evaluated in this study. The concentration levels, expressed in picograms per milliliter, were calculated with RayBiotech Q Analyzer software against a standard curve set for each biomarker. The total amount of each biomarker was determined in picograms by timing the concentration with sample volume.

Statistical Analysis

Data was analyzed with GraphPad prism software, version 5 (GraphPad Software Inc, La Jolla, Calif). Nonparametric tests were used to compare the differences between groups and sites ($P < .05$). The Kruskal-Wallis and Pearson's chi-square tests were used to compare differences between the three groups in each region for quantitative and qualitative comparisons, respectively. When there was a significant difference, a multiple-comparison post hoc test (Dunn's test) was used to determine the pairwise differences. The Wilcoxon signed rank test was used to compare differences between sites (incisor versus premolar) within each group for quantitative variables. Fisher's exact and McNemar's tests were used for the same purpose for the comparison of qualitative variables between the sites.

RESULTS

Table 1 presents the mean demographic data, as well as the mean clinical parameters and GCF volume, for the three groups. Age and gender distribution did not differ significantly between clinical groups. The

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Table 2. Concentration and Total Amount of Biomarkers[a] by Group and Site

		Concentration (pg/mL)						Total Amount (pg)					
		MMP-9		IFN-γ		IL-10		MMP-9		IFN-γ		IL-10	
		IC*	PM	IC	PM*	IC	PM*	IC*	PM	IC	PM*	IC	PM
Group 1	Mean	16,644	21,341	14.4	18.2	2.2	2.7	2.17	3.58	0.0021	0.0021	0.00029	0.00027
	SD	13,327	12,446	9.07	11.48	3.1	2.9	1.85	3.05	0.0017	0.0010	0.00047	0.00025
	Median	20,300	23,210	16.3	16.85	0	1.65	2.43	2.77	0.0015	0.0022	0	0.00026
Group 2	Mean	2990	10,850	181	2469	14.9	354	0.49	2.01	0.0204	0.3500	0.0016	0.0515
	SD	5230	7905	473	7134	36.8	990	0.93	2.05	0.0521	0.9979	0.0040	0.1384
	Median	1028	12,700	0.9	200	0	35.1	0.14	1.57	0.0001	0.0259	0	0.0049
Group 3	Mean	14,512	18,315	3.9	78.7	1.4	25.9	5.02	3.88	0.0005	0.0232	0.00021	0.0088
	SD	9447	14,511	4.9	126	2.1	48.5	7.97	3.35	0.0010	0.0567	0.00046	0.0227
	Median	14,620	15,450	1.6	21.8	0.3	8.35	1.86	2.47	0.0002	0.0024	0.00003	0.00055

[a] IC indicates incisor region; PM, premolar region; MMP-9, matrix metalloproteinase-9; IFN-γ, interferon-gamma; and IL-10, interleukin-10.
* Statistically significant difference between groups ($P \leq .05$).

fixed retainer group exhibited more PA in the incisor region ($P = .03$). In addition, the GCF volume was higher in the incisor region of group 1 than in the other groups, but this difference was not statistically significant. No significant differences between groups were observed for the remaining clinical parameters.

Table 2 provides the concentration and total amount of each biomarker by group and site. The concentration of certain biomarkers differed significantly in the GCF between the three groups; significant differences between groups were detected for IL-10, IFN-γ, and MMP-9. The median concentrations of IL-10 and IFN-γ were significantly higher in the premolar sites of group 2 patients (lower removable retainers) ($P = .04$ and $P = .01$, respectively), whereas the median concentration of MMP-9 was significantly higher ($P = .02$) in the GCF samples collected from the lower incisors of group 1 patients (fixed retainers) (Figure 2). The total amount of MMP-9 at incisor sites differed significantly between the three groups ($P = .017$), as did the total amount of IFN-γ at premolar sites ($P = .012$). No statistically significant results were observed for the total amount of IL-10 at both premolar and incisor sites (Table 2).

Although our data showed a fairly consistent trend toward increased concentrations of the targeted biomarkers in GCF samples obtained from premolar sites, a significant difference between the two sites was found only in group 2 (Table 3). Interestingly, in patients with removable retainers, the median concentrations of IL-10 (35.1 pg/mL), IFN-γ (200 pg/mL), and MMP-9 (12,700 pg/mL) in premolar sites were significantly higher when compared to the incisor sites (Figure 3).

DISCUSSION

It is reasonable to state that the clinical periodontal health of our subjects was not affected by bonded lingual retainers, despite the fact that significantly increased PA was found in the lower incisor region in group 1 (Table 1). Störmann and Ehmer[16] also found that patients with fixed retainers had increased PA over a 24-month follow-up period, although regular oral hygiene instruction was given to the patients. In addition, our study confirms and extends the results of Heier et al.,[5] who found no differences in gingival inflammation in spite of slightly increased PA in

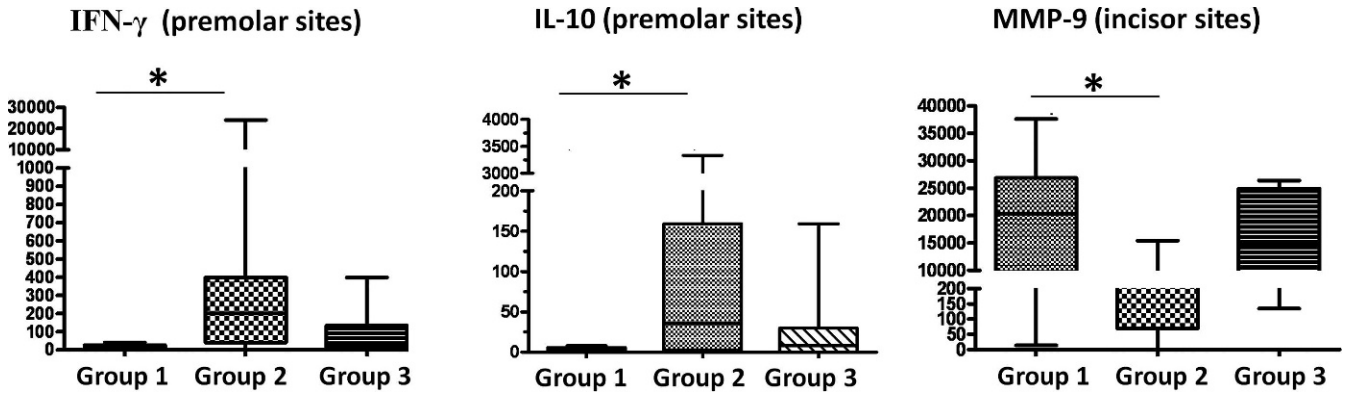

Figure 2. Box plots displaying biomarker concentrations in GCF samples obtained from premolar and incisor sites of each group. *Significant differences between groups.

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Table 3. Comparison of Biomarker Concentrations[a] (Median Values, Expressed in pg/mL) Between Sites Within Each Group

	Site A (IC)	Site B (PM)	*P*
Group 1			
IL-10	0	1.65	NS (.45)
IFN-γ	16.3	16.85	NS (.57)
MMP-9	20,300	23,210	NS (.31)
Group 2			
IL-10	0	35.1	.016*
IFN-γ	0.9	200	.015*
MMP-9	1028	12,700	.018*
Group 3			
IL-10	0.3	8.35	NS (.17)
IFN-γ	1.6	21.8	NS (.14)
MMP-9	14,620	15,450	NS (.7)

[a] Site A (IC) indicates incisor region; Site B (PM), premolar region; MMP-9, matrix metalloproteinase-9; IFN-γ, interferon-gamma; and IL-10, interleukin-10.

* Statistically significant at $P \leq .05$. NS indicates non–statistically significant differences.

patients with fixed retainers during the first 6 months of retention.

Few studies[5,17] have investigated the impact of removable retainers on periodontal health. Heier et al.[5] evaluated the differences between fixed and removable retainers, but only the segment from canine to canine was analyzed. The present study featured a more comprehensive evaluation since a premolar site was also included in the study design. One important finding is the fact that group 2 was the only group to exhibit statistically significant intragroup differences, with a higher concentration of biomarkers in the premolar sites versus the incisor sites (Figure 3). There are two possible explanations for this finding. Perhaps the posterior acrylic resin portion of the removable retainer irritates the periodontal tissues, or perhaps the GCF levels of biomarkers are elevated in the premolar region as a result of tooth movement induced by the removable retainer. The results from previous studies,[18–20] which have shown elevated expression of MMP-9, IL-10, and IFN-γ in the periodontium during orthodontic movement, together with the results from the current study indicating that periodontal clinical parameters (PA, PD, and BOP) in premolar sites are not different between groups, support the latter. Nevertheless, the two possibilities are by no means mutually exclusive.

Not only periodontal disease but also tooth movement can induce differential expression of cytokines in the periodontium. Garlet et al.[19] demonstrated that IL-10 is up-regulated in the tension zone, whereas Alhashimi et al.[18] found higher IFN-γ expression in the compression zone during orthodontic tooth movement. As a result, it is possible that the different biomarker profile found at premolar sites under the influence of removable retainers may be a consequence of relapse or natural adaptation of the occlusion in the posterior segment of the arch. The fixed retainer extends from canine to canine only, whereas the removable retainer evaluated in our study is the Hawley-type, which extends distally up to the molar region. Since group 2 patients were wearing retainers only at night, it is possible that the appliance was constantly moving those teeth back to their original position after a day of posterior teeth drifting caused by the lack of retainer wear. As a result, we may hypothesize that the posterior teeth are constantly under light pressure during nighttime removable retainer wear, and this may explain the elevated concentrations of biomarkers in the GCF collected from premolar sites in group 2. It was intriguing to observe that the same behavior was not observed in the anterior region of the removable retainer group, despite the fact that lower incisor movement is the most common manifestation of orthodontic relapse.[1] This may help provide a better understanding of the mechanism of action of lower removable retainers. From our biological observations, we can speculate that the posterior segment of the lower arch is a critical area, where the removable retainer counteracts the forces that act on the teeth throughout the day, thus

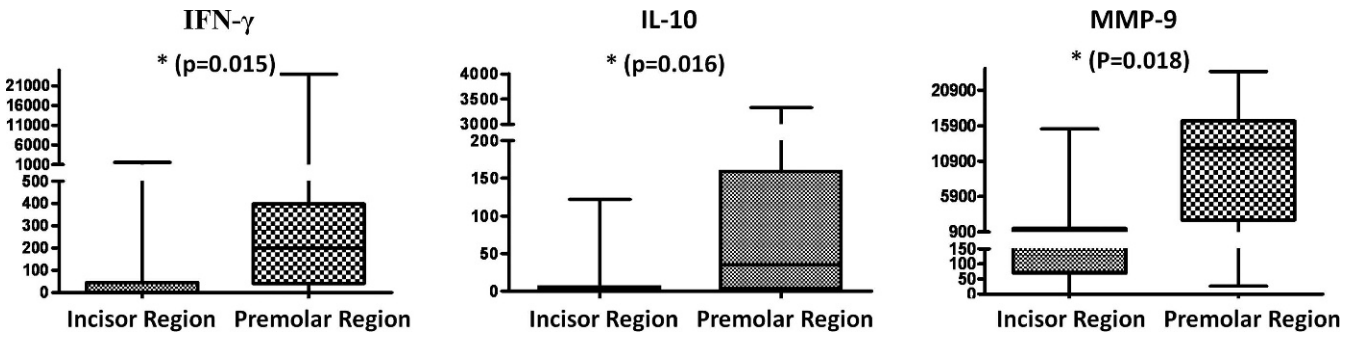

Figure 3. Box plots displaying biomarker concentrations in GCF samples obtained from premolar and incisor sites of group 2. *Significant differences between sites.

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avoiding anterior crowding by preventing mesial drifting of the posterior teeth.

Our research confirms the findings of previous studies[3–6] that found no relationship between fixed retainers and periodontal disease. However, it was interesting to observe the significantly elevated concentration of MMP-9 associated with a higher percentage of sites with visible plaque in the incisor region of the fixed retainer group. It is also important to note that there was a trend toward an increased volume of GCF in the incisor region of group 1, although this was not statistically significant (Table 1). MMP-9 activities from polymorphonuclear neutrophil sources have been shown to be markedly increased in human gingival tissue, GCF, and saliva.[9,20,21] This means that dental plaque may trigger the release of MMPs in GCF, regardless of clinical inflammation. It has to be stated that the mere elevation of MMP-9 in periodontally healthy sites of patients using fixed orthodontic retainers may or may not be a risk factor for future periodontal bone loss in the incisor region. A recent study[22] supports the biological plausibility of an association between GCF levels of MMP-9 and progression of periodontitis in immunocompromised patients; however, further studies are needed to validate MMP-9 as a prognostic factor for periodontitis in healthy individuals. Despite the difficulties and uncertainties in this field of research, it should be kept in mind that high loads of MMP-9 may be a complicating factor with potentially longer orthodontic retention periods.

Although promising, the study of GCF composition is still a challenging research field. The sensitivity of the assay methods has improved since research on the constituents of GCF first began; nevertheless, it is still far from being ideal. The main limitation of the present study resides in the small number of subjects in each group, which, along with the asymmetry of the data sets, may have resulted in a lack of statistical power. In fact, the inclusion criteria were an important limiting factor in terms of sample size for group 2, because most patients were not wearing removable retainers on a daily basis after some years in retention. In addition, measurement of biomarkers in GCF is not an easy task because the volumes of GCF are usually low.

CONCLUSIONS

- The data indicated specific alterations in the GCF composition as a result of the presence of different orthodontic retainers.
- Increased GCF levels of MMP-9 from lower incisor sites in the fixed retainer group suggested subclinical inflammation.
- The elevated concentrations of IL-10 and IFN-γ at premolar sites in the removable retainer group may be related to the restraining effect exerted by the appliance on the posterior segment of the arch.

ACKNOWLEDGMENT

This work was funded by the University of Manitoba Research Grants Program.

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