Original Article

Metal and Ceramic Bracket Effects on Human Buccal Mucosa Epithelial Cells

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ABSTRACT

Objective: To test the null hypothesis that metal and ceramic brackets have no effect on the epithelial cells of the buccal mucosa.

Materials and Methods: Two metal and two ceramic brackets were bonded in 21 individuals of both sexes. With the use of liquid-based exfoliative cytology, morphometric and morphologic changes in buccal mucosa cells adjacent to these brackets were determined and were compared at three time points: baseline (T0), 60 days after placement (T1), and 30 days after removal of the brackets (T2).

Results: A decrease in nuclear area and an increase in cytoplasmic area occurred in the buccal mucosa cells adjacent to the brackets at T1 ($P < .01$). At T2, this altered morphometry persisted only in cells adjacent to the metal brackets, although to a lesser degree than at T1 ($P < .01$). A greater decrease in nuclear area was noted in cells adjacent to the metal brackets than in those next to the ceramic brackets ($P < .01$). At T0, the proportions of surface and subsurface cells were similar, but at T1, a predominance of surface cells was observed ($P < .05$). At all time points, smears of cells appeared normal or normal with some inflammatory changes.

Conclusion: The hypothesis is rejected. Placement of metal and ceramic brackets in the buccal cavity induces cellular alterations. These alterations do not suggest malignancy. (*Angle Orthod.* 2008;00:373–379.)

KEY WORDS: Metal bracket; Ceramic bracket; Buccal mucosa; Epithelial cells

INTRODUCTION

Placement of orthodontic appliances in a healthy oral cavity can induce a continuous accumulation of dental plaque,[1] alter the normal oral microbiota,[2,3] cause lesions in the buccal mucosa,[4] exacerbate periodontal disease, and consequently cause infection.[5] It is known that ulceration in the buccal mucosa is one of the most frequent complaints of patients because of the friction between bracket and mucosa, which causes discomfort for the patient.[6,7] Thus, when these ulcerations persist during treatment, the orthodontist refers the patient to another specialist, who performs additional tests such as taking a biopsy and doing exfoliative cytology, which can detect alterations in the buccal mucosa caused by this irritation.

The use of exfoliative cytology in the diagnosis of buccal lesions was more common during the period from 1955 to 1975. Since then, a decline in its clinical application has occurred because of the subjective nature of its interpretation, and because few abnormal cells can be identified in smears. However, this technique has stirred renewed interest because of the possibility of its being complemented with other laboratory techniques such as molecular biology, cytomorphology, and immunohistochemistry.[8] In addition, it offers the advantage of being minimally invasive and painless, without the need for local anesthetic, and it is easy to perform.

Another major advance in cytopathology was the

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development of liquid-based cytology, which provides a series of advantages in relation to the conventional type, featuring (1) better evidence of epithelial cells, (2) slides with fewer inflammatory cells and red blood cells, less cell debris, and fewer undesirable artifacts,[9] (3) less cell overlapping, and (4) more representative samples for reading.[10] Because liquid-based cytology mixes the complete sample in liquid, probably fewer false negatives occur, compared with conventional cytology, wherein only 20% of collected cells are transferred to the slide.[9]

The necessity of offering patients a fixed orthodontic treatment with no significant risk of damage to buccal mucosa cells and the lack of reports in the literature on the cytologic analysis of buccal mucosa adjacent to metal and ceramic brackets were the reasons for this study.

The aim of this investigation was to study and compare the epithelial cells of the buccal mucosa adjacent to metal and ceramic brackets at three time points: baseline, 60 days after placement, and 30 days after removal of the brackets. These cells were examined for morphometric alterations in the area of the nucleus and cytoplasm, alterations in the nuclear/cytoplasmic ratio, morphologic alterations in the nucleus and cytoplasm, and alterations in the cytologic criteria for malignancy.

MATERIALS AND METHODS

Individuals who were referred to the Dental Clinic for dental treatment were invited to participate in this study before beginning their treatment. When they agreed to participate, individuals or their legal guardians signed an informed consent form.

Selected individuals had no related history of smoking, alcoholism, diabetes, anemia, or debilitating diseases and were not being treated with antibiotics or steroids during the study period. They did not use alcohol-based mouthwashes, did not wear prostheses or have tooth restorations with sharp edges, and did not have any type of lesion on the buccal mucosa. The sample of this study comprised 21 Brazilian individuals (mean age, 14 years; range, 7.6 to 53.7 years)—7 males and 14 females.

The locations chosen for bracket placement were second deciduous molar, second premolar, and first and second permanent molars, all on the upper arch. The teeth chosen varied according to the stage of dentition for each at the time of bracket placement.

Four premolar brackets were bonded in each individual with Transbond XT adhesive (3M Unitek Orthodontic Product, Monrovia, Calif). Two were standard edgewise Dyna-lock metal brackets (3M Unitek Orthodontic Product) placed on the right side, and two were standard edgewise Transcend Series 6000 ceramic brackets (3M Unitek Orthodontic Product) placed on the left side (Figures 1 and 2).

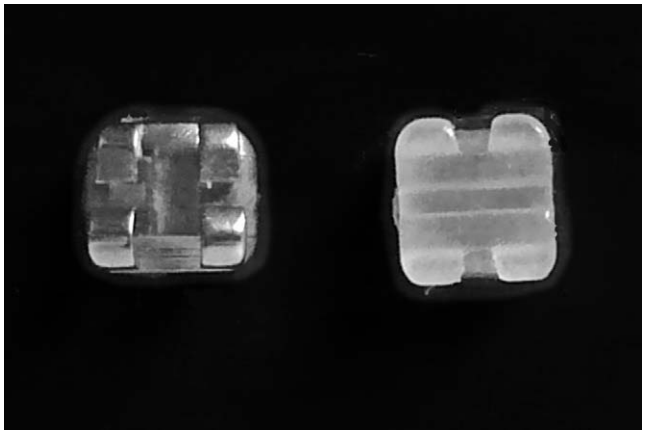

Figure 1. Dyna-lock metal bracket and Transcend Series 6000 ceramic bracket in frontal view.

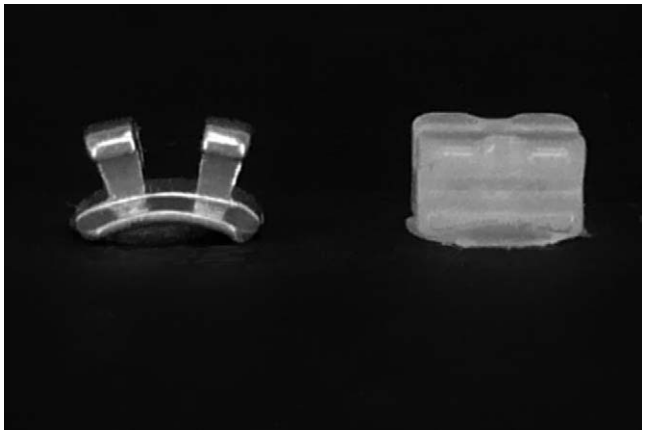

Figure 2. Dyna-lock metal bracket and Transcend Series 6000 ceramic bracket in occlusal view.

Liquid-Based Cytology

Epithelial cells were collected at three times by the same operator: baseline (T0), 60 days after placement (T1), and 30 days after removal of the brackets (T2). T0 was used as a control, and cells were collected from areas of clinically healthy buccal mucosa.

Before cell collection, individuals were instructed to rinse the mouth with water to remove possible debris. Cells were collected with a DNA-Citoliq System kit (Digene Brasil LTDA, São Paulo, Brazil), called the Universal Collection Medium (UCM). The collected material was immersed in liquid medium in the kit flask, where it remained until the histologic process had been completed. The slides were stained with the use of a Papanicolaou technique that was modified according to the manufacturer's recommendations for the DNA-Citoliq System (2002).

Cytomorphometric Analysis

The slides were examined under a binocular light microscope (Olympus BX50, Olympus, Tokyo, Japan) adapted with WH 10X-H/22 oculars and a PLAN 40X/0.65 objective (Olympus). Prior to reading, the identification number of the slides was covered to avoid bias. Fifty cells on each slide were selected randomly for examination.[11–14] Areas with cells folded over and/or clumped were avoided[15] because of the difficulty involved in determining cell boundaries. The image of the cytologic fields was captured at a magnification of 400 times (Figures 3A through E) with the use of a Sony CCD Iris Color Video camera, model DXC-107A (Sony Electronics Inc, Tokyo, Japan). To measure nuclear (NA) and cytoplasmic (CA) areas, the image analysis system Image Pro Plus, version 4.5.029 for Windows 98/NR/2000 (Media Cybernetics Inc, Silver Spring, Md), was used to enhance precision and speed of measurement.[16] After NA and CA were measured,[11,12] the nuclear/cytoplasmic ratio (N/C) was determined for each cell.[11,14,17]

Cytomorphologic Analysis

Cytomorphologic analysis was performed with the use of the same binocular microscope adapted with WH 10X-H/22 oculars and PLAN 10X/0.25, 20X/0.40, and 40X/0.65 objectives (Olympus). The slide was scanned completely, and the smears were classified on the basis of the predominance of cells present, in accordance with the method of Sugerman and Savage.[18]

The smears also were evaluated qualitatively, according to the cytologic criteria of malignancy, and were classified according to Papanicolaou[19] as follows:

Class 0: Material insufficient or inadequate for analysis
Class I: Smear normal
Class II: Smear normal with inflammatory changes
Class III: Dysplastic changes—smear suspect
Class IV: Strongly indicative but not conclusive for malignancy
Class V: Smear malignant

Statistical Analysis

A two-way analysis of variance (ANOVA) ($P < .01$) with repeated measurements was used to determine whether there was a significant difference for NA, CA, and N/C between the various time points. When ANOVA noted a difference between groups, Tukey's Honestly Significant Differences (HSD) test was used ($P < .01$).

For comparison of cell morphology for the different times and brackets, McNemar's test for significance of changes was used ($P < .05$).

RESULTS

Cytomorphometric Analysis

After placement of the brackets, a significant decrease in NA and N/C and an increase in CA of buccal mucosa cells adjacent to ceramic or metal brackets were observed ($P < .01$). Cells adjacent to metal brackets did show a lower NA and N/C compared with those next to ceramic brackets ($P < .01$). Although a greater increase was noted in the CA of cells adjacent to metal brackets than in those next to the ceramic bracket, it was not significant ($P > .05$).

When the brackets were removed, the buccal mucosa cells adjacent to metal brackets still showed a smaller nucleus, a larger cytoplasm, and a lower nuclear/cytoplasmic ratio than at T0 ($P < .01$), although with fewer alterations than at T1 ($P < .01$). On the other hand, the buccal mucosa cells adjacent to the ceramic brackets returned to their initial size ($P > .05$) (Tables 1 through 3).

Cytomorphologic Analysis

With respect to the predominance of cells present in the smears, on the basis of their staining, no slide was found with a predominance of cells of the spinosum and basale stratum.

In T0, the number of slides with a predominance of surface and subsurface cells was similar, and for T1 and T2, a greater number of slides showed a predominance of surface cells. A statistically significant difference was found between T0 and T1 in the buccal mucosa cells adjacent to the ceramic and metal brackets, and between T0 and T2 in the buccal mucosa cells adjacent to the ceramic brackets ($P < .05$) (Table 4).

Smears that were examined exhibited no instances of Papanicolaou Classes 0, III, IV, and V when the cytologic criteria for malignancy were determined. Classes I and II were observed at all time points, and no significant difference was noted among the groups ($P > .05$).

DISCUSSION

In this study, ceramic and metal brackets were bonded without the presence of arches, ligatures, or rubber bands because these materials could bias the findings.[20] However, it is known that these accessories are used routinely in treatment, and they can protect the buccal mucosa from direct friction caused by the bracket.

Ulcerations in the buccal mucosa are frequent complaints among orthodontic patients. Studies indicate

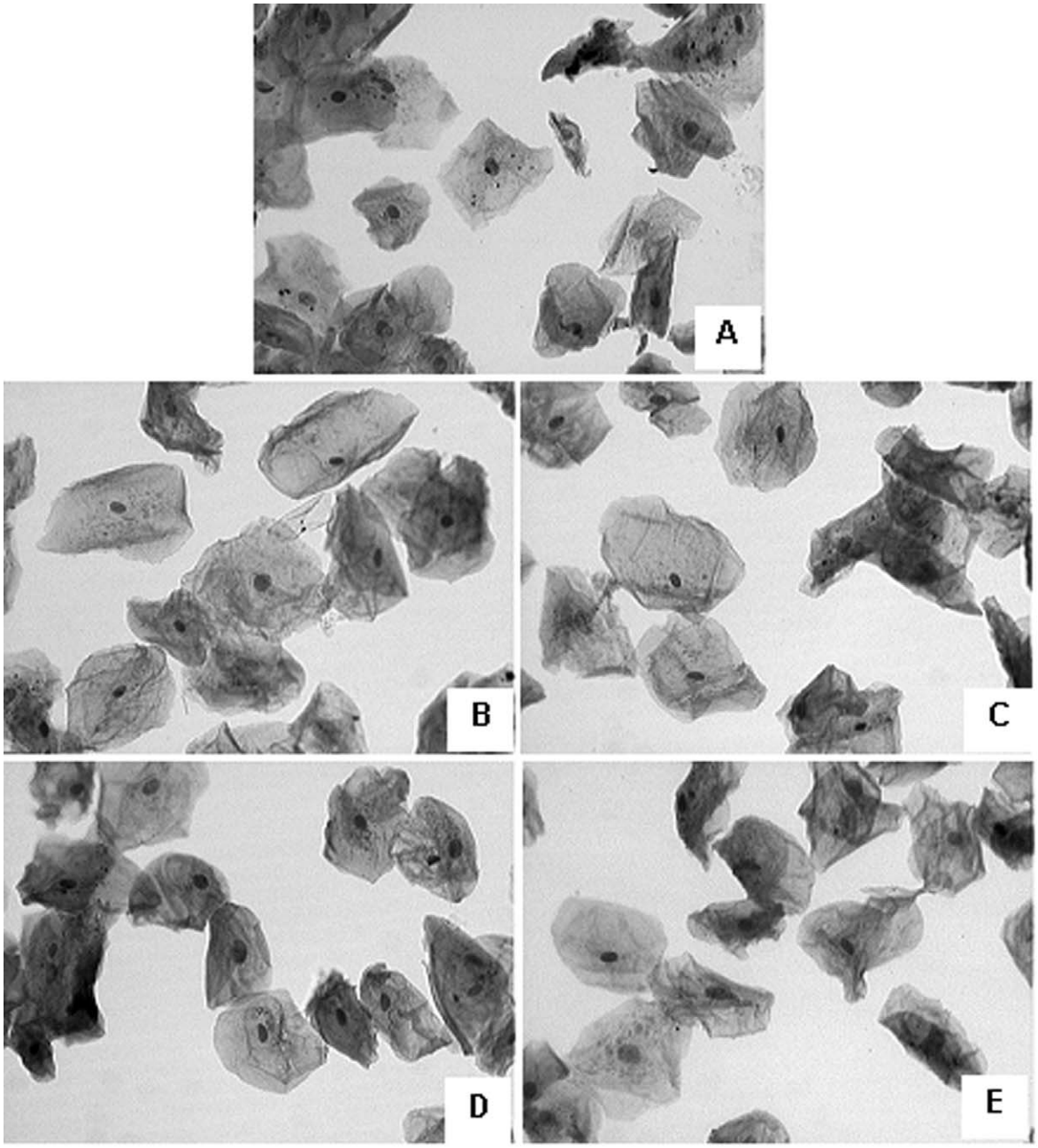

Figure 3. (A) Epithelial cells of clinically healthy buccal mucosa at T0. (B) Epithelial cells of buccal mucosa adjacent to the metal brackets at T1. (C) Epithelial cells of buccal mucosa adjacent to the ceramic brackets at T1. (D) Epithelial cells of buccal mucosa adjacent to the metal brackets at T2. (E) Epithelial cells of buccal mucosa adjacent to the ceramic brackets at T2 (Papanicolaou, 400×).

Table 1. Nuclear Area According to Bracket and Time

	T0	T1 Ceramic	T1 Metal	T2 Ceramic	T2 Metal
Mean	63.39 μm^2	57.92 μm^2	53.00 μm^2	63.69 μm^2	59.45 μm^2
Standard deviation	±19.23	±19.79	±17.37	±20.89	±20.30
T0		0.00002*	0.00002*	0.99655	0.00003*
T1 ceramic	0.00002*		0.00002*	0.00002*	0.34155
T1 metal	0.00002*	0.00002*		0.00002*	0.00002*
T2 ceramic	0.99655	0.00002*	0.00002*		0.00002*
T2 metal	0.00003*	0.34155	0.00002*	0.00002*	

* $P < .01$ indicates significant difference.

Table 2. Cytoplasmic Area According to Bracket and Time

	T0	T1 Ceramic	T1 Metal	T2 Ceramic	T2 Metal
Mean	2015.1 μm^2	2406.1 μm^2	2473.9 μm^2	2041.4 μm^2	2155.4 μm^2
Standard deviation	±696.75	±642.18	±621.03	±639.44	±677.04
T0		0.00002*	0.00002*	0.87705	0.00002*
T1 ceramic	0.00002*		0.1041	0.00002*	0.00002*
T1 metal	0.00002*	0.1041		0.00002*	0.00002*
T2 ceramic	0.87705	0.00002*	0.00002*		0.00040*
T2 metal	0.00002*	0.00002*	0.00002*	0.00040*	

* $P < .01$ indicates significant difference.

Table 3. Nuclear-Cytoplasmic Ratio According to Bracket and Time

	T0	T1 Ceramic	T1 Metal	T2 Ceramic	T2 Metal
Mean	0.0341	0.0253	0.0224	0.0336	0.0296
Standard deviation	±0.01	±0.01	±0.01	±0.01	±0.01
T0		0.00002*	0.00002*	0.81662	0.00002*
T1 ceramic	0.00002*		0.00002*	0.00002*	0.00002*
T1 metal	0.00002*	0.00002*		0.00002*	0.00002*
T2 ceramic	0.81662	0.00002*	0.00002*		0.00002*
T2 metal	0.00002*	0.00002*	0.00002*	0.00002*	

* $P < .01$ indicates significant difference.

Table 4. Number of Slides According to Cell Predominance

Variables	Superficial	Sub-superficial	Spinosum	Basale
T0	10	11	0	0
T1 ceramic	17	4	0	0
T1 metal	19	2	0	0
T2 ceramic	18	3	0	0
T2 metal	16	5	0	0

that approximately 76%[6] to 95%[7] of patients report ulcers in the buccal mucosa during treatment, and only between 16.5%[7] and 21.1%[6] of patients report ulcers only once. Therefore, because the epithelium of the buccal covering is exposed to aggressive agents, as in the case of brackets that are capable of causing alterations at various times during treatment, exfoliative cytology can be an effective tool in diagnosis to detect and evaluate these alterations, assuming that its limitations are well elucidated and applied.[21,22] The clinician should be knowledgeable about this technique because the cells are studied individually and cannot be evaluated with regard to tissue conformation, as in a biopsy.[21] In addition, only the most surface cells of the epithelium are collected for exfoliative cytology.[23] Therefore, the use of brushes to collect epithelial cells allows collection of samples that include cells of all the buccal mucosa stratified squamous epithelium,[9,24,25] in addition to providing a thinner and more dispersed, homogeneous distribution of cells on the slides.[26]

In the present study, placement of brackets in the buccal cavity caused diminution of the nucleus, an increase in cytoplasm, and a lower nuclear/cytoplasmic ratio of buccal mucosa cells that were in contact with the brackets. These results corroborate the findings of Shabana et al,[27] who also reported a statistically significant increase in the size of cells of traumatic keratosis lesions when these were compared with normal cells of the buccal mucosa.

However, in the buccal cells of individuals with malignant lesions[11,13] or of smokers,[12,15] alterations distinct from those in the present study were found. In individuals with a tobacco-chewing habit and in those with smoking and tobacco-chewing habits combined, an increase in nuclear diameter and a decrease in cell diameter were observed,[15] as were seen in samples of individuals with tumors in the mouth floor.[13] Cowpe et al[11] did not find size changes in the nucleus in samples of suspicious lesions of the buccal mucosa and the mouth floor but did observe a decrease in cytoplasmic area in lesions of the buccal mucosa. Ogden et al[12] observed an increased nuclear area only in the buccal mucosa cells of smokers and did not note an alteration in the cytoplasmic area. Normal cells of the buccal mucosa have abundant cytoplasm and a single, small centralized nucleus; malignant cells have a broad, enlarged nucleus that occupies a large area of the cytoplasm, with well-stained chromatin and an irregular nuclear membrane.[21] Therefore, the cellular changes that occurred in the buccal mucosa adjacent to the metal and ceramic brackets in the present study do not suggest malignancy. When this diagnosis was confirmed by the evaluation of cytologic criteria for ma-

lignancy, smears of only Classes I and II of Papanicolaou were noted.

Alterations in sizes of the nucleus and cytoplasm as demonstrated here suggest hyperkeratinosis of the stratified squamous epithelium of the buccal mucosa adjacent to the brackets. This would cause an increase in the number of cells in the corneum stratum of the epithelium that show abundant cytoplasm and smaller nuclei than cells from deeper layers. This hyperkeratinosis can be confirmed by an increase in the number of slides with a predominance of surface cells at T1 and T2.

Greater cell alterations on the side with the17-4 stainless steel bracket may have been caused by trauma to the buccal mucosa caused by the physical characteristics of brackets, in other words, because of the fact that the wings were less rounded than those of the ceramic brackets, or because of the cytotoxicity of stainless steel, which has been observed in other studies.[28–32]

In this study, buccal mucosa cells were evaluated only 30 days after removal of the brackets, because Jones et al[22] recommend that if a lesion persists for longer than 14 days after removal of the causative factors, a biopsy should be performed immediately. Therefore, within 30 days, cells should have returned to their initial size. In future studies, the buccal mucosa cells should be analyzed after longer periods to determine whether these alterations persist in the buccal mucosa.

This was the first study undertaken to describe cellular changes in the buccal mucosa adjacent to metal and ceramic brackets. Prior to this finding, orthodontists had no way of defending against allegations that linked the use of brackets to oral cancer. Because brackets are essential components of fixed orthodontic appliances, biocompatibility is needed to prevent irreversible deleterious damage to tissues. Although results of this investigation suggest that brackets do not cause any malignant changes in the buccal mucosa, the origin of the observed changes remains uncertain. Future studies in this regard will explore ways to prevent these alterations.

CONCLUSIONS

- Placement of metal and ceramic brackets in the buccal cavity induces cellular alterations. These alterations do not suggest malignancy.
- Buccal mucosa cells adjacent to the metal brackets show greater changes than are seen in those adjacent to the ceramic brackets.
- Buccal mucosa cells adjacent to the metal and ceramic brackets tend to return to the initial morphology after removal of the brackets.

REFERENCES

1. Sukontapatipark W, El-Agroudi MA, Selliseth NJ, Thunold K, Selvig KA. Bacterial colonization associated with fixed orthodontic appliances: a scanning electron microscopy study. *Eur J Orthod.* 2001;23:475–484.
2. Batoni G, Pardini M, Giannotti A, Ota F, Gluca MR, Gabriele M, Campa M, Senesi S. Effect of removable orthodontic appliances on oral colonization by mutans streptococci in children. *Eur J Orthod.* 2001;109:388–392.
3. Hägg U, Kaveewatchranont P, Samaranayake YH, Samaranayake LP. The effect of fixed orthodontic appliances on the oral carriage of *Candida* species and Enterobacteriaceae. *Eur J Orthod.* 2004;26:623–629.
4. Bishara SE. Oral lesions caused by an orthodontic retainer: a case report. *Am J Orthod Dentofacial Orthop.* 1995;108: 115–117.
5. Atack NE, Sandy JR, Addy M. Periodontal and microbiological changes associated with the placement of orthodontics appliances: a review. *J Periodontol.* 1996;67:78–85.
6. Kvam E, Gjerdet NR, Bondevik O. Traumatic ulcers and pain during orthodontic treatment. *Community Dent Oral Epidemiol.* 1987;15:104–107.
7. Kvam E, Bondevik O, Gjerdet NR. Traumatic ulcers and pain in adults during orthodontic treatment. *Community Dent Oral Epidemiol.* 1989;17:154–157.
8. Macluskey M, Ogden GR. An overview of the prevention of oral cancer and diagnosis markers of malignant change: 2 markers of value in tumour diagnosis. *Dent Update.* 2000; 27:148–152.
9. Kahn MA. Oral exfoliative cytology procedures: conventional, brush biopsy and ThinPrep. *J Tenn Dent Assoc.* 2001; 81:17–20.
10. Hayama FH, Motta ACF, Silva APG, Migliari DA. Liquid-based preparations versus conventional cytology: specimen adequacy and diagnostic agreement in oral lesions. *Med Oral Patol Oral Cir Bucal.* 2005;10:115–122.
11. Cowpe JG, Longmore RB, Green MW. Quantitative exfoliative cytology of abnormal oral mucosal smears. *J R Soc Med.* 1988;81:509–513.
12. Ogden GR, Cowpe JG, Green MW. Quantitative exfoliative cytology of normal buccal mucosa: effect of smoking. *J Oral Pathol Med.* 1990;19:53–55.
13. Mollaoglu N, Cowpe JG, Walker R. Cytomorphologic analysis of Papanicolaou stained smears collected from floor of the mouth mucosa in patients with or without oral malignancy. *Turk J Med Sci.* 2001;31:225–228.
14. Alberti S, Spadella CT, Francischone TRCG, Assis GF, Cestari TM, Taveira LAA. Exfoliative cytology of the oral mucosa in type II diabetic patients: morphology and cytomorphometry. *J Oral Pathol Med.* 2003;32:538–543.
15. Ramaesh T, Mendis BRRN, Ratnatunga N, Thattil RO. The effect of tobacco smoking and of betel chewing with tobacco on the buccal mucosa: a cytomorphometric analysis. *J Oral Pathol Med.* 1999;28:385–388.
16. Ogden GR, Cowpe JG, Wight AJ. Oral exfoliative cytology: review of methods of assessment. *J Oral Pathol Med.* 1997; 26:201–205.
17. Cowpe JG, Longmore RB, Green MW. Quantitative exfoliative cytology of normal oral squames: an age, site and sex-related survey. *J R Soc Med.* 1985;78:995–1004.
18. Sugerman PB, Savage NW. Exfoliative cytology in clinical oral pathology. *Aust Dent J.* 1996;41:71–74.
19. Lange DE, Meyer M, Hahn W. Oral exfoliative cytology in diagnosis of viral and bullous lesions. *J Periodontol.* 1972; 43:433–437.

20. Kluemper GT, Hiser DG, Rayens MK, Jay MJ. Efficacy of a wax containing benzocaine in the relief of oral mucosal pain caused by orthodontic appliances. *Am J Orthod Dentofacial Orthop.* 2002;122:359–365.
21. Bernstein ML, Miller RL. Oral exfoliative cytology. *J Am Dent Assoc.* 1978;96:625–629.
22. Jones AC, Migliorati CA, Stewart CM. Oral cytology: indications, contraindications, and technique. *Gen Dent.* 1995; Jan–Feb:74–77.
23. Folsom TC, White CP, Bromer L, Canby HF, Garrington GE. Oral exfoliative study: review of the literature and report of a three-year study. *Oral Surg Oral Med Oral Pathol.* 1972; 33:61–74.
24. Sciubba JJ. Improving detection of precancerous and cancerous oral lesions: computer-assisted analysis of the oral brush biopsy. U.S. Collaborative OralCDx Study Group. *J Am Dent Assoc.* 1999;130:1445–1457.
25. Mehrotra R, Gupta A, Singh M, Ibrahim R. Application of citology and molecular biology in diagnosing premalignant or malignant oral lesions. *Mol Cancer.* 2006;23:5–11.
26. Jones AC, Pink FE, Sandow PL, Stewart CM, Migliorati CA, Baughman RA. The cytobrush plus cell collector in oral cytology. *Oral Surg Oral Med Oral Pathol.* 1994;77:101–104.
27. Shabana AHM, El-Labban NG, Lee KW, Kramer IRH. Morphometric analysis of suprabasal cells in oral white lesions. *J Clin Pathol.* 1989;42:264–270.
28. Faccioni F, Franceschetti P, Cerpelloni M, Fracasso ME. In vivo study on metal release from fixed orthodontic appliances and DNA damage in oral mucosa cells. *Am J Orthod Dentofacial Orthop.* 2003;124:687–694.
29. Grimsdottir MR, Hensten-Pettersen A, Kullmann A. Cytotoxic effect of orthodontic appliances. *Eur J Orthod.* 1992;14: 47–53.
30. Grimsdottir MR, Hensten-Pettersen A. Cytotoxic and antibacterial effects of orthodontic appliances. *Scand J Dent Res.* 1993;101:229–231.
31. David A, Lobner D. *In vitro* cytotoxicity of orthodontic archwires in cortical cell cultures. *Eur J Orthod.* 2004;26:421–426.
32. Oh KT, Choo SU, Kim KM, Kim KN. A stainless steel bracket for orthodontic application. *Eur J Orthod.* 2005;27:237–244.