Clinical Research

The Effect of Bleaching Systems on the Gingiva and the Levels of IL-1β and IL-10 in Gingival Crevicular Fluid

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Clinical Relevance

Home and chemically activated bleaching systems could be considered safer in tooth whitening and maintaining gingival health when compared with a light-activated bleaching system.

SUMMARY

Objective: This study aimed to evaluate the color change and clinical periodontal parameters and to analyze the interleukin-1 beta (IL-

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 1β) and interleukin-10 (IL-10) levels in gingival crevicular fluid (GCF) of patients treated with different bleaching systems.

Materials and Methods: According to pre-established criteria, 30 healthy volunteers were selected and randomly divided into three groups (n=10): G1, home bleaching (Opalescence 35% Carbamide Peroxide, CP); G2, chemically activated office bleaching (Opalescence Xtra Boost 38% Hydrogen Peroxide, HP); G3, light-activated office bleaching (Opalescence Xtra 35% HP). Treatments were applied according to the manufacturer's recommendations. After shade evaluation, clinical periodontal parameters were evaluated as follows: gingival index (GI), plaque index (PI), and bleeding on probing (BOP). GCF were collected from six maxillary sites per patient at baseline (T0), one day (T1) after bleaching treatments, and 15 days (T2) after bleaching treatments and analyzed for IL-1ß and IL-10 by

enzyme-linked immunosorbent assay. Data were subjected to statistical analysis (p < 0.05).

Results: Spectrophotometer readings exhibited significant differences among the groups (p < 0.05). The ΔE values (color change) of G3 were statistically higher than the other groups (p < 0.05). The PI of G3 after 15 days was significantly higher than the PI of G2 after 15 days (p < 0.05). The GI of G2 was lower than that of G1 and G3 before bleaching (p < 0.05). According to BOP, no significant differences were found among the groups at any time intervals (p>0.05). In G3, the total amount of IL-1ß after 15 days was higher than the amount before bleaching (p < 0.05). The IL-10 total amount and concentration levels did not exhibit any significant differences among the groups or by time (p>0.05).

Conclusion: Home and chemically activated bleaching systems could be considered as safer in tooth whitening and maintaining gingival health when compared with a light-activated bleaching system, which might lead to increased proinflammatory cytokine (IL-1 β).

INTRODUCTION

Bleaching has been universally accepted as a method of lightening discolored teeth, and several vital bleaching systems have been introduced in response to the demand in esthetic dentistry.¹⁻³ This procedure consists of hydrogen peroxide or carbamide peroxide gel applications that can be done by the dentist in office or by the patient at home.^{2,3} Although the at-home bleaching system is the most frequently recommended treatment for vital teeth, some patients do not want to use a bleaching tray or do not like to wait two weeks to see the result of their treatments. These patients might request a method that produces more immediate results, the in-office bleaching treatment.^{3,4} The use of light sources has been recommended to accelerate the action of the bleaching gel in in-office bleaching.^{1,3,5–8}

The evaluation of the efficacy and safety of bleaching systems has recieved considerable attention.^{1,4,9,10} Although generally positive results have been reported concerning the whitening ability of these agents, concerns still remain as to their effects on periodontal tissues.^{11,12} Since their use, significant controversy has been generated regarding the risk of tissue damage from the contact of bleaching agents with the oral mucosa. Local side effects on oral soft tissues caused by peroxides released into the oral cavity from bleaching systems are reported in the literature as gingival irritations such as gum burning or erosions of the gingiva.¹²⁻¹⁴

Many critical interactions among the cells of the immune system are controlled by soluble mediators called cytokines.^{15,16} These regulatory molecules can regulate not only local and systemic immune and inflammatory response but also wound healing, hematopoesis, and many other biologic processes. As in other chronic inflammatory diseases such as rheumatoid arthritis, cytokines are considered to play an important role in the initiation, progression, and host modulation of periodontal diseases.^{15–18}

Interleukin-1 beta (IL-1 β) is of particular interest since it is a proinflammatory, multifunctional cytokine, which promotes bone resorption and stimulates eicosanoid production.^{16,17,19} IL-1 β also participates in many aspects of the immune response and has been shown to be present and elevated in the tissues and gingival creviculer fluid (GCF) of patients with periodontal disease. IL-1 β is a key mediator of the host inflammatory and tissue regulatory pathways in a number of chronic inflammatory disorders, such as periodontitis.^{16,17,19}

The anti-inflammatory cytokines are a series of immunoregulatory molecules that control the proinflammatory cytokine response.^{16,17} Interleukin-10 (IL-10), also known as anti-inflammatory cytokinelike IL-4, is secreted by T lymphocytes, monocytes, keratinocytes, and activated B cells and can exihibit regulatory effects on macrophages, neutrophils, natural killer cells, B cells, and mast cells. The secretion of IL-1, IL-6, TNFa, and IL-8 is also blocked by IL-10, which can at the same time regulate humoral immune response by suppressing T-cell functions and stimulating B-cell differentation.^{16,17,20}

Investigating the effect of bleaching systems on GCF pro-/anti-inflammatory response and gingival health may provide important data about the effects of treatment models on gingival tissues.²¹ Thus, the aim of this study was to evaluate the color change and clinical periodontal parameters and to analyze IL-1 β and IL-10 levels in the GCF of patients treated with different bleaching systems.

MATERIALS AND METHODS

Thirty voluntary patients (7 male and 23 female) in good oral and general health having a desire to lighten their maxillary and mandibular anterior teeth participated in this study. The Ethics Committee of Hacettepe University, Ankara, Turkey, re-

viewed and approved the research protocol and informed consent form. All patients received a professional dental prophylaxis two weeks before the start of bleaching treatments and were asked to brush their teeth twice daily to standardize tooth cleaning during the study. All subjects had anterior baseline color shade of at least A3 or darker on the Vita Shade Guide (VITAPAN classical, VITA Zahnfabrik, Bad Sackingen, Germany). Inclusion criteria were the presence of all natural teeth (intact dentition); the absence of tooth sensitivity, caries, restoration, and periodontal disease; no previous bleaching treatment; the absence of smoking habits; and compliance with instructions to avoid staining from food and beverages during the treatment period.

Participants between 18 and 37 years of age (average, 23.7 years) were randomly assigned to three groups of 10 volunteers according to the bleaching systems (Table 1) and provided their written consent and completed medical history forms prior to the start of the study.

Group 1 was treated with an at-home bleaching system (Opalescence 35% carbamide peroxide [CP], Ultradent, Salt Lake City, UT, USA), group 2 was treated with a chemically activated office bleaching system (Opalescence Xtra Boost 38% hydrogen peroxide [HP], Ultradent), and and group 3 was treated with a light-activated bleaching system (Opalescence Xtra 35% HP, Ultradent). Each bleaching system was applied to both maxillary and mandibular arches according to the manufacturer's recommendations. All teeth were cleaned using pumice and a slow-speed rotary brush/prophy cup 24 hours before the bleaching applications.

Group 1—Maxillary and mandibular alginate impressions were made and dental stone models were cast for fabrication of bleaching trays. Ethyl-vinyl-acetate trays were made with a heat/vacuum tray–

forming machine, and the trays were trimmed to fit each model perfectly. Patients were instructed on how to care for and use the trays correctly and were asked to wear their trays filled with the bleaching gel (Opalescence 35% CP, Ultradent) for a half-hour each day for two weeks. Participants returned every seven days for an oral examination and interview regarding symptoms.

Group 2—The gingival tissues were isolated using a light-cured resin dam (Opal Dam, Ultradent) to prevent the bleaching gel from contacting the gingival tissues. The bleaching gel (Opalescence Xtra Boost 38% HP, Ultradent) was provided with two syringes: one syringe contained the activator while the other contained hydrogen peroxide. Before use, the activator was mixed with the bleaching agent. The activated gel was applied to the teeth for 15 minutes with two applications. At the end of the bleaching treatment, the barrier was removed and the treated teeth were thoroughly rinsed with airwater spray for 30 seconds.

Group 3—In this group, after the gingival tissues were isolated using a light-cured resin dam (Opal Dam, Ultradent), the bleaching agent (Opalescence Xtra 35% HP, Ultradent) was applied twice for 15 minutes. An LED lamp (Biowhite Accelerator, Ensodent, Pistoia, Italy) was used to activate the bleaching agent during each gel application (15 minutes times two). At the end of bleaching treatment, the Opal Dam barrier was removed from surfaces, and the treated teeth were thoroughly rinsed with air-water spray for 30 seconds.

The groups were examined and evaluated at baseline (T0), the day after bleaching treatments (T1), and 15 days after bleaching treatments (T2). Color change was determined using a digital spectrophotometer (Vita Easy Shade, Vident, Brea, CA, USA) for comparing each bleaching treatment to baseline.¹ The color was measured on the facial

Table 1: Bleaching Systems Used in the Study									
Product	Manufacturer	Concentration, %	Duration of Bleaching	Activation					
Opalescence PF	Ultradent Dental GmbH, Salt Lake City, UT, USA	35 CP	30 min/d for 2 wk	—					
Opalescence Xtra Boost	Ultradent Dental GmbH, Salt Lake City, UT, USA	38 HP	2 imes 15 min	Chemical					
Opalescence Xtra	Ultradent Dental GmbH, Salt Lake City, UT, USA	35 HP	2 imes 15 min	Light activation (BioWhite Accelerator, Ensodent, Italy)					

surfaces of 10 maxillary teeth (central, lateral incisors, cuspids, and premolars) by one experienced and qualified examiner. Three spectrometer readings were made with the active point of the instrument focused on the middle third of each tooth. A mean color for each tooth and patient were accordingly calculated. Intraoral photographs (DSC-D770, Sony, Tokyo, Japan) were also taken to record the tooth shade for documentation of the baseline shade and for comparison after treatment.

Collection of GCF

Maxillary teeth were selected for sampling to eliminate the possibility of contamination with saliva. GCF samples were collected from six maxillary sites per patient. The strips obtained from each patient were studied as a pooled sample. Sampling teeth were isolated with cotton rolls and gently dried. A standard paper strip (Perio-paper, IDE Interstate, Amityville, NY, USA) was inserted into the sulcus to the depth of 1-2 mm for 30 seconds, and the strips were moved immediately to a calibrated Periotron 8000 (Oraflow Inc, Smith-town, NY, USA) to determine the GCF volume (Figures 1 and 2). Strips contaminated by blood were excluded from the sampled group. After collection of the gingival fluid, the strips were immediately placed in sterile Eppendorf tubes containing 10 mM NaH₂PO₄ and 150 mM NaCl, pH 7.2, followed by mixing and centrifugation at 800g. The GCF samples were stored at -80°C until subsequent analysis.¹⁶

Six sites for each anterior maxillary tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual, and distolingual) were examined and scored for clinical parameters including plaque index (PI),



Figure 1. GCF collection with a standard paper strip.

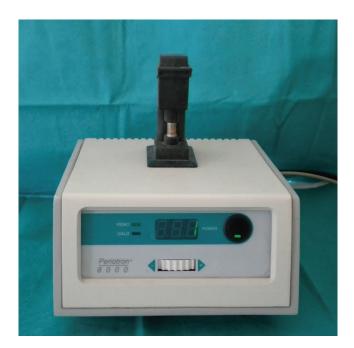


Figure 2. The Periotron 8000 device used in this study.

gingival index (GI), and bleeding on probing (BOP).^{21–25} To eliminate the risk of interfering with the actual GCF quality and quantity, clinical measurements were recorded after GCF sampling. To increase reliability, all clinical measurements were performed by the same examiner. All samples were obtained in the morning following an overnight fast. Subjects were asked not to drink (except water) or chew gum for the same period, and abstention was checked prior to GCF sample collection.²⁶ For standardization, all GCF samples were obtained between 9:00 AM and 10:00 AM. The baseline GCF sampling and clinical measures were performed one week after cleaning of all teeth using pumice and a slow-speed rotary brush/prophy cup. In addition, GCF sampling and clinical measurements were repeated the day after (T1) and 15 days after (T2)bleaching treatments.

Assays of IL-1β and IL-10

GCF samples were analyzed for IL-1 β and IL-10 using commercially available enzyme-linked immunosorbent assays (ELISAs; R&D System Inc., Minneapolis, MN, USA). Analyses were performed according to the manufacturer's protocol. All ELISA determinations were performed in duplicate. Results were calculated using the standard curves created in each assay. The total amount of cytokines in GCF was determined in picograms (pg).

Table 2:Color Change (ΔL , Δa , Δb , and ΔE Values), Mean (SD)									
Groups (N=10)	ΔL	Δа	Δb	ΔΕ					
G1 (Opalescence PF)	5.0 (0.163)	-1.2 (0.013)	-1.4 (0.043)	5.32 (0.152)					
G2 (Opalescence Xtra Boost)	5.1 (0.130)	-1.1 (0.012)	-1.5 (0.010)	5.42 (0.086)					
G3 (Opalescence Xtra)	5.2 (0.141)	-1.3 (0.010)	-1.6 (0.158)	5.59* (0.174)					
Abbreviations: ΔE , $[(L_1 - L_2)^2 + (a_1 - a_2)^2$ the lightness/value; .	$(b_1 - b_2)^2 j^{1/2}$; a, depicts the c	chromacity in the red-green axis; b	o, depicts the chromacity in the yea	llow-blue axis; L, depicts					

* Significantly different from the other two groups (p < 0.5).

Statistical Analysis

The normality of data distribution and the homogeneity of group variances were verified prior to selection of test methods.

The color change results were then submitted to parametric statistical analysis using one-way analysis of variance.

The data for clinical parameters and IL levels were subjected to statistical analysis for differences among and within the groups with nonparametric tests, since the data distribution was not normal. The differences among the groups for the assessed criteria at each time were evaluated using Kruskal-Wallis followed by Mann-Whitney Utest with Bonferroni correction. The differences by the time (T0, T1, T2) within each group were determined by Friedman test followed by Wilcoxon signed ranks test with Bonferroni correction. The changes between time points (T0-T1 and T0-T2) were also evaluated to be able to compare the effects of bleaching treatments using the Kruskal-Wallis test. In all of the tests, the level of significance was set at p < 0.05, and calculations were handled by the SPSS 12.0 software for Windows (SPSS Inc, Chicago, IL, USA).

RESULTS

There were significant differences among the ΔE levels of the groups (p < 0.05). The G3 showed the highest color change (p < 0.05; Table 2).

At baseline, there were no statistically significant differences among the groups regarding the parameters assessed (p < 0.05) except GI, which was lower in G2 (0.09 ± 0.1) than G1 and G3 (p < 0.05).

The PI of G3 after 15 days was significantly higher (0.16 \pm 0.18) than the PI of G2 after 15 days (p < 0.05; Table 3).

According to BOP, no significant differences were found among the groups at any time intervals (p>0.05; Table 3).

In G3, the total amount and concentration of IL-1 β after 15 days was higher than the amount before

Time	Groups (N=10)										
	G1	(Opalescence	PF)	G2 (Op	alescence Xtra	Boost)	G3 (Opalescence Xtra)				
	то	T1	T2	то	T1	T2	то	T1	T2		
GI	0.28 (0.17)	0.27 (0.22)	0.2 (0.18)	0.09* (0.1)	0.09 (0.08)	0.21 (0.48)	0.35 (0.28)	0.22 (0.21)	0.26 (0.21)		
PI	0.08 (0.13)	0.05 (0.14)	0.07 (0.12)	0.04 (0.06)	0.04 (0.07)	0.03 (0.09)	0.16 (0.19)	0.09 (0.11)	0.16 ^{**} (0.18)		
BOP	0	0	0	0	0	0	0	0	0		

* Significantly different from the other groups at T0.

** Significantly different from G2 at T2.

	Groups (N=10)										
	G1	(Opalescence	PF)	G2 (Opa	alescence Xtra	a Boost)	G3 (Opalescence Xtra)				
	Т0	T1	T2	ТО	T1	T2	ТО	T1	T2		
IL-1β Total	58.9 (93.06)	40.96 (58.42)	33.5 (43.1)	23.03 (35.27)	25.8 (38.6)	22.6 (31.4)	46.92 (95.89)	72.2 (102.6)	72.9* (100.7)		
IL-1β Concentration	4.38 (2.55)	4.54 (1.99)	4.54 (2.8)	3.59 (2.67)	3.35 (2.2)	2.61 (1.73)	3.99 (3.06)	4.67 (2.51)	5.19* (3.53)		
IL-10 Total	0.005 (0.009)	0.003 (0.006)	0.003 (0.004)	0.003 (0.06)	0.003 (0.004)	0.001 (0.002)	0.004 (0.008)	0.003 (0.004)	0.004 (0.005		
IL-10 Concentration	10.72 (14.14)	9.19 (11.54)	6.56 (6.57)	5.82 (5.77)	6.5 (7.3)	6.57 (7.11)	13.34 (20.76)	16.49 (20.02)	19.04 (23.27		

bleaching (p < 0.05; Table 4). There were no significant differences among the total amounts and concentrations of both IL levels before bleaching, one day after bleaching, and 15 days after bleaching

in G1 and G2 (p>0.05). The IL-10 total amount and concentration levels did not exhibit any significant differences among the

groups or by the time (p>0.05).

The changes in clinical parameters (GI, PI, and BOP) and IL-1 β and IL-10 concentration levels between the T0-T1 and T0-T2 time points did not significantly differ according to bleaching treatments (Table 5; p>0.05).

DISCUSSION

This study compared the efficacy and side effects of three different bleaching systems used clinically with different bleaching protocols. A number of methods are available for evaluating the efficacy of bleaching. Shade guides, photography, colorimeter spectrophotometer, or computer digitization can be used to assess tooth color changes. Although the use of value-oriented standard shade guides is the most common clinical method,²⁷ the selection of the matching shade tab is subjective, not predictably reproducible, and influenced by such factors as lighting and eye fatigue. Since the spectrophotome-

Table 5: Changes in Clinical Parameters and IL-1 β and IL-10 Levels at T1 and T2 According to Bleaching Treatments, Mean (SD)

Groups (N=10)	GI		PI		BOP		IL-1β Concentration		IL-10 Concentration	
	T0-T1	T0-T2	T0-T1	Т0-Т2	T0-T1	то-т2	T0-T1	T0-T2	T0-T1	T0-T2
G1 (Opalescence PF)	0.02 (0.18)	0.08 (0.15)	0.03 (0.03)	0.02 (0.1)	0	0	-0.16 (1.73)	-0.16 (2.25)	1.529 (12.763)	4.153 (10.836)
G2 (Opalescence Xtra Boost)	-0.01 (0.11)	-0.13 (0.46)	0.01 (0.07)	0.02 (0.07)	0	0	0.24 (1.74)	0.98 (3.01)	-0.686 (7.392)	-0.755 (6.414)
G3 (Opalescence Xtra)	0.13 (0.16)	0.09 (0.17)	0.08 (0.19)	0.01 (0.19)	0	0	-0.68 (3.45)	-1.20 (2.77)	-3.144 (6.931)	-5.703 (14.428)
p	0.144	0.243	0.575	0.924	-	-	0.792	0.241	0.337	0.440
Abbreviations: To, before bleaching; T1, one day after bleaching; T2 15 days after bleaching ($p < 0.05$).										

ters give more objective results than shade tabs do,⁶ the color changes were evaluated using this method in the current study. Although the results obtained by such a method may not always correlate with the clinical outcomes, it was our intention to determine the differences in the bleaching efficacy of the systems in a more sensitive and standardized way.

Shade changes evaluated with the spectrophotometer showed that there was a significant difference in the overall color change (ΔE) with all three techniques, but the light-activated office bleaching group (G3) had the highest shade change. Spectrophotometer readings were in line with previous reports.⁸ However, it is difficult to compare the results of this study with data from the literature, as there are no published studies available in which these three systems were compared with each other using the same methodology.

Bleaching has been reported to cause a number of side effects.^{1,4,9,10,12,13,21} The most common side effects are tooth hypersensitivity and gingival irritation.^{1,10,28} These effects are correlated with several factors, such as pH and the peroxide concentration of the bleaching gel, the number and length of applications, and other factors. All of the observed and most of the reported oral adverse effects are mild and transient in nature.^{1,11,28}

It is also discussed in the literature whether peroxides from bleaching gels function as a disinfectant consistent with their application in periodontal treatments or if they enhance inflammation and irritation of periodontal cells and structures.^{13,21} In an animal study evaluating the influence of CP on gingival structures, CP was reported to cause an augmentation in the proliferative activity within the basal and parabasal layers of the gingival epithelium, resulting in a change in tissue morphology.²⁹

Therefore, this study was motivated in part by the concerns expressed by the American Dental Association regarding the potential adverse effects of peroxide-containing agents on gingival tissues and the PI, GI, and BOP levels before, one day after, and 15 days after bleaching treatments were evaluated. Using these standard clinical parameters, it was possible to evaluate the status of gingival tissues, those immediately adjacent to the bleaching agents before and after bleaching procedures.^{16,21–25}

Hannig and others¹¹ examined the bleeding index of the patients treated with two different bleaching systems and observed a significant decrease for both groups during the bleaching therapy. Leonard and others⁹ evaluated the changes in the GI of the patients treated with different concentrations of HP and CP and concluded no significant changes after bleaching treatment.

Tavares and others⁷ also measured the gingival health of three patients treated with 15% HP with the use of light by examining the GI and PI at baseline, immediately posttreatment, at three months, and at six months. Although signs of mild irritation were seen in all groups immediately after treatment, no significant differences between groups were noted. The GI of all groups decreased significantly after therapy with no change in PI. The GI in all treatment groups was significantly less than baseline at three months. The GI reduction of the peroxide and light subjects was significantly less than that of the lightalone treatment group at six months.

The findings of this study indicated no significant differences in BOP levels either among the different bleaching systems or by the time. This might be the result of the good fit of the tray, the shorter application duration compared with the other commercially available agents, the neutral pH of the agent for the at-home bleaching system, and the close professional control in the application and protection of surrounding tissue as an integral part of the procedure for office bleaching systems used in this study. Although there were no statistically significant differences in PI levels within each group at any time, the PI of the light-activated office bleaching group (G3) after 15 days was higher than that of the chemically activated office bleaching group (G2).

Clinical parameters are very important in evaluating the condition of periodontal tissues; however, they are not sensitive enough to assess the inflammatory changes at a cellular level.^{11,14,16,22–25,30} They are limited to identifying qualitative changes in gingival tissues. Examination of GCF and its constituents was excepted as a better, more current, and more sensitive method for evaluating gingival inflammation.^{16,19,21} The present study was unique as there have been no published data to date about the effect of bleaching on cytokine levels in GCF.

The intensity and duration of inflammation, severity of bone loss, and the resolution of an inflammatory state depend on shifting the balance between the activities of proinflammatory and anti-inflammatory cytokines during the periodontal inflammation.^{15,16,18,31} In this study, the proinflammatory IL-1 β and anti-inflammatory IL-10 levels in the GCF of patients treated with different bleaching systems were evaluated.

Numerous studies have reported increased IL-1 β levels in the GCF from periodontally diseased sites compared with healthy sites.^{16,18,25,30–32} These data suggest that the total amount of IL-1 β in GCF was elevated with increasing inflammation. The results of this study showed that no significant differences were found between the home bleaching (G1) and chemically activated office bleaching (G3) groups regarding the amount of IL-1 β at any time interval. The total amount of IL-1 β levels significantly increased for the light-activated office bleaching group (G3) after 15 days, which might be due to the heat propagated during light activation. However, it is important to note that this study was carried out three years ago, and the light-activated office bleaching used in the study was withdrawn from the dental market by the manufacturer.

IL-10 was reported to increase with increasing inflammation and to play an important role in the pathogenesis of gingivitis and periodontitis, especially by down-regulating the production of monocyte-derived proinflammatory cytokines such as IL- 1β .^{17,18,31,33} The findings of the current study showed no significant difference in IL-10 levels either among the groups or by the time, indicating the absence of any anti-inflammatory response triggered by different bleaching systems.

It is also important to perform some histological studies including oral soft-tissue epithelia and possible DNA alterations to verify that these results are reproducible and consistent. Thus, further research with a larger population and different bleaching systems would help in clarifying the possible side effects of bleaching on surrounding soft tissues.

CONCLUSION

- 1. Home and chemically activated bleaching systems might be considered safer treatment choices in maintaining gingival health.
- 2. Since a significant increase was observed in proinflammatory cytokine IL-1 β levels without any change in anti-inflammatory IL-10 levels with the light-activated bleaching system used in this study, light-activated bleaching might be thought to have a negative effect on gingival health by changing the anti-inflammatory-proinflammatory cytokine ratio.
- 3. Further studies are needed to unravel the role of bleaching systems on the production of cytokines in maintaining gingival health.

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Announcements



Erratum

In Operative Dentistry 36(6) 572–580, The Effect of Bleaching Systems on the Gingiva and the Levels of IL-1 β and IL-10 in Gingival Crevicular Fluid an author name was omitted from the list of authors. Dr. Ozlem Ozer Yucel should be listed as 4th co-author with the following affiliation address: Özlem Özer Yücel, DDS, PhD., Department of Oral Pathology, Faculty of Dentistry, Gazi University, Ankara, Turkey. The Authors and Operative Dentistry apologize for this omission.