Research Article

The effects of overhang amalgam restorations on levels of cytokines, gingival crevicular fluid volume and some periodontal parameters

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Abstract: Purpose: To evaluate clinical periodontal findings and GCF levels of tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6) and interleukin 8 (IL-8) in patients with overhang amalgam restorations before and after overhang restoration removal. **Methods:** 22 volunteer subjects (age range: 22-42 years old) with 22 overhang Class II amalgam restorations were selected. GCF samples were obtained from adjacent and contralateral teeth for IL-6, IL-8 and TNF-α measurements and analyzed using enzyme-linked immunosorbent assay (ELISA) kits. The amalgam overhangs were removed and the readings were repeated at the end of 1 week. Clinical periodontal assessments, including gingival index (GI) and plaque index (PI) were performed at baseline and after 1 week. Data were analyzed using the paired t test and independent sample t-test at a significance level of 0.05. Correlations were investigated using Pearson correlation analysis. **Results:** A significant reduction in GI and PI was observed after removal of the overhanging restoration on Day 7. ($P < 0.05$) However, there was no significant difference between IL-6 ($P = 0.857$), IL-8 ($P = 0.579$) and TNF-α ($P = 0.958$) levels before and after overhang restoration removal. There were no significant correlations between laboratory findings and clinical parameters. (*Am J Dent* 2016;29:266-270).

Clinical significance: Dental restorations may produce periodontal disease and may alter tooth form, surface integrity, relationship with the periodontium and adjacent teeth. The marginal edge located in the gingival sulcus is likely the cause of the inflammatory reaction. This study showed significant improvement in clinical periodontal parameters after removing the overhangs of restorations.

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Introduction

The relationship between dental restorations and periodontal tissues has been investigated over many years. Studies on the prevalence of overhangs,[1,2] the effect of overhangs,[3,4] and periodontal tissues/restorative material interaction[5] are available.

The most important mechanism by which dental restorations may affect marginal health is the enhancement of plaque accumulation. Overhanging margins provide ideal locations for plaque accumulation and result in a change in the ecological balance of the gingival sulcus region, thereby causing an increase in the amount of disease-associated microorganisms.[3,6-8] Poorly contoured restorations can increase plaque retention and/or violate the biological width.[9] The placement of interproximal restorations predisposes the adjacent periodontal tissues to future breakdown.[4,10] A significantly greater prevalence and degree of severity of periodontal disease may be associated with the presence of overhanging restorations. Removal of an overhang has been shown to result in improved plaque control and restoration of gingival health.[11]

Gingival crevicular fluid (GCF) can be collected from the gingival sulcus surrounding the teeth and exists as either a serum transudate or, more commonly, as an inflammatory exudate. GCF contains substances from the host as well as from microorganisms in the subgingival and supragingival plaque. GCF contains metabolic and bacterial products such as lactic acid, urea, hydroxyproline, endotoxin, cytotoxic substances, and hydrogen sulfide. Constituents from the host include molecules from blood, and contributions from cells and tissues of the periodontium. The latter includes the vasculature, epithelium, and non-mineralized and mineralized connective tissues, as well as inflammatory and immune cells that have infiltrated the periodontal tissues. Among the important host-derived constituents in GCF are markers of inflammation, including enzymes and cytokines. Further products of tissue breakdown can also be detected in crevicular fluid.[12-14]

Cytokines are low molecular weight proteins synthesized in response to bacteria and their products, inducing and maintaining an inflammatory response. ‘‘Cell stressors’’ of non-microbial origin may also stimulate synthesis of cytokines.[14] A large number of cytokines present in GCF are potentially useful diagnostic or prognostic markers of periodontal destruction.[15,16] Various proinflammatory mediators, such as IL-1, IL-8, IL-6 and TNF-α, have been widely studied in the GCF, gingival tissue and serum of healthy gingivitis and chronic periodontitis patients.[17-20] IL-6 and IL-8 function in concert with other members of the cytokine network in order to regulate the cellular inflammatory response in the periodontium.[21] IL-8 is produced by a wide variety of cells (polymorphonuclear leukocytes, monocytes, macrophages and fibroblasts) and plays a key role in the accumulation of leukocytes at sites of inflammation. TNF-α is a potent immunological mediator with pro-inflammatory properties and is secreted by monocytes and macrophages. It also induces the secretion of collagenase by fibroblasts and the resorption of cartilage and bone. It has also been implicated in the destruction of periodontal tissue during periodontal disease.[22]

To the best of our knowledge, no reports have been published concerning the effect of overhanging amalgam restorations on the GCF levels of IL-6, IL-8 and TNF-α. Therefore, this study compared the short-term clinical changes

Table 1. Paired sample t-test for different time periods.

	TNF-α (pg/mL)	IL-6 (pg/mL)	IL-8 (pg/mL)	GFC volume (µL)	PI	GI
Control Baseline	5.81±0.18	130.81±14.16	156.46±14.11	0.22±0.05	0.63±0.15	0.32±0.15
Control 7 days	5.87±0.15	131.03±13.57	116.48±10.24	0.19±0.02	0.61±0.12	0.48±0.16
Mean change, SD	-0.06±0.64	-0.21±5.43	39.98±15.31	0.03±0.20	0.02±0.26	-0.16±0.41
P value	0.741	0.884	0.483	0.514	0.807	0.168
Exp. Baseline	6.09±0.43	98.31±11.14	130.29±14.02	0.27±0.04	1.29±0.16	1.11±0.18
Exp. 7 days	6.11±0.23	100.94±9.77	119.97±8.91	0.35±0.09	0.51±0.12	0.43±0.11
Mean change, SD	-0.021±1.51	-2.62±5.35	10.32±16.77	-0.09±0.30	0.78±0.41	0.68±0.56
P value	0.958	0.857	0.579	0.297	0.001*	0.002*

*P< 0.05.

occurring in the periodontium and changes in cytokine levels in GCF after the removal of overhanging amalgam in subgingival restorations.

Materials and Methods

Patients and study design - Twenty-two volunteers (13 females and 9 males; mean age 35.0 years, age range 22-42) attending restorative dentistry clinics at the Faculty of Dentistry, Ataturk University, Turkey, participated in the study. The Ataturk University Ethics Committee granted ethical approval (29.04.2009/021) for each assessment phase. Overhang amalgam restorations, which were detected during routine 6-month dental examinations according to patient compliance and radiographic results, were included in this study. Subjects gave informed consent before participating. Personal information related to subjects' medical and dental history was obtained.

Inclusion criteria – Inclusion criteria were: at least one previously done overhang Class II amalgam restoration with subgingival margins including the occlusal surface and mesial or distal surfaces, good general health, no use of anti-inflammatory drugs in the 2 months preceding the study, no antibiotic therapy within the previous 6 months, good periodontal health, with generalized probing depths ≤ 3 mm and no radiographic evidence of periodontal bone loss. To be considered for the study, restorations had to have been made previously and detected with radiographic and clinical examination.

Exclusion criteria – Subjects reporting cigarette smoking, diabetes, immuno-compromise, pregnancy and breast-feeding women, use of orthodontic devices, ongoing dental or periodontal treatment 12 months prior to the beginning of the study or use of antibiotics within 6 months prior to clinical examination or medication that might lead to decreased salivary flow, were excluded from participation in the study.

Plaque Index (PI),[22] Gingival Index (GI),[23] and gingival crevicular fluid (GCF) were analyzed before amalgam restorations were removed. Overhang amalgam restorations were removed using an aerator straight bur. The cavity was checked radiographically and clinically to determine whether the removal of the overhang restoration was successful. Dycal[a] was placed in the deep parts and amalgam restorations replaced. All restorations were performed by the same operator. All study subjects were motivated to maintain good oral hygiene.

Periodontal examination and GCF sampling - Radiographic examination and clinical periodontal assessments, including GI[23] and PI,[24] were performed at baseline and the end of the experiment. The same trained examiner (SM) recorded PI and GI at the overhang dental surface using a periodontal probe. The reproducibility of these measurements using k statistics resulted in k = 0.42.

GCF was collected from the mesial or distal surface of teeth with class II overhang restorations as the experimental group. The remaining natural teeth in the opposite arch acted as controls. After PI was assessed, supragingival plaque was removed. The sites to be sampled were isolated with cotton rolls, carefully sprayed with water to remove saliva and finally gently dried with an air syringe. A saliva ejector was used to avoid salivary contamination. A paper strip (Periopaper[b]) was inserted into the intracrevicular space 1 mm below the gingival margin and left in place for 30 seconds. Three paper strips (one for each mediator to be measured) were taken from the overhang restoration and three from the contralateral tooth. Six paper strips were sampled for each subject. Strips contaminated with blood were discarded. GCF, PI and GI were reassessed 1 week later. The strips were transferred for volume determination to a chairside electronic gingival fluid measuring device (Periotron 8000[c]) calibrated using known volumes of phosphate-buffered saline. The paper strips were then immediately placed into three labeled Eppendorf tubes (Microcentrifuge tubes[d]) containing 300 µl of 0.9% physiological saline solution, isolated with ParafilmH M[e] to avoid evaporation and sent to the laboratory. After 15 minutes of shaking at room temperature, the strips were removed and the eluates centrifuged (20 minutes, ×3,000 g) to remove plaque and cellular elements. The samples were stored at -80°C for subsequent assays. The procedure was repeated 1 week after replacement of the restorations.

Analysis of cytokine production - Cytokine assay levels of IL-6, IL-8 and TNF-α in samples were determined using appropriate commercial ELISA kits[f] according to the manufacturer's instructions. The results were read using a microplate reader at a wavelength of 405 nm. Levels of IL-6, IL-8 and TNF-α were expressed as pg/mL. Data were analyzed using SPSS[g] 16.0 for Windows and first examined for normality using the Kolmogorov-Smirnov test. Since the data were normally distributed, comparisons between the mean values of two groups were performed using the paired t- test and independent sample t-test. In all tests, P< 0.05 was regarded as statistically significant. Pearson's correlation test was used to determine the relationships between clinical and laboratory parameters.

Results

For cytokine levels in GCF (pg/mL) and clinical parameters measured, paired sample t-test was used to evaluate statistically

Table 2. Independent sample t test for experimental and control site.

	TNF-α (pg/mL)	IL-6 (pg/mL)	IL-8 (pg/mL)	GFC volume (μL)	PI	GI
Control Baseline	5.81±0.18	130.81±14.16	156.46±14.11	0.22±0.05	0.63±0.15	0.32±0.15
Exp. Baseline	6.09±0.43	98.31±11.14	130.29±14.02	0.27±0.04	1.29±0.16	1.11±0.18
P value	0.560	0.083	0.206	0.502	0.005*	0.003*
Control 7 days	5.87±0.15	131.03±13.57	116.48±10.24	0.19±0.02	0.61±0.12	0.48±0.16
Exp. 7 days	6.11±0.23	100.94±9.77	119.97±8.91	0.35±0.09	0.51±0.12	0.43±0.11
P value	0.400	0.084	0.801	0.075	0.527	0.789

*P< 0.05.

significant differences between pre- and post-operative period. Means and standard deviations are presented in Table 1 (*P< 0.05).

The independent sample t-test was used for comparison of cytokine levels in GCF and clinical parameters values between experimental and control groups. Means and standard deviations are presented in Table 2 (*P< 0.05).

A significant reduction in GI and PI was observed after removal of the overhanging restoration on Day 7 (P< 0.05). However, there was no significant difference between amounts of IL-6, IL-8 and TNF-α before and after removal of overhang restorations. Although there was a rise in GCF volume on Day 7 after removal of overhang restoration in the experimental group, this was not statistically significant (P> 0.05).

Comparison of baseline measurements between the control and experimental groups revealed higher PI and GI values in the overhang restoration group (P< 0.05). Comparison between the groups at the end of Day 7 revealed no significant difference in any parameters.

There were no significant correlations between laboratory findings and clinical parameters.

Discussion

Overhang restorations may have negative effects on gingival and periodontal tissues. This clinico-biochemical pilot study was designed to estimate the clinical periodontal parameters, GCF volume and GCF levels of cytokines in patients with overhang amalgam restorations before and after restorative treatment, as well as to evaluate any correlation between these parameters.

An overhanging dental restoration is primarily found in Class II restorations, since the placement of interdental restorations is often difficult even for enhanced wedge and matrix systems. We therefore selected patients with Class II overhang amalgam restorations.

There are many ways of collecting GCF, including paper strips, capillary tubes, paper cones and gingival washing. The present study used paper strips, as employed in other studies.[4,25,26] GCF is an exudate derived from a variety of sources, including microbial dental plaque, host inflammatory cells, host tissue and serum. The amount of this fluid tends to increase with inflammation and capillary permeability.[27]

A number of methods have been described for assessment of GCF volume such as colorimetry (ninhydrin or fluroscein dye), weighing and use of an electronic machine (Periotron).[28] For this study, the Periotron was used as it is an electronic system that calculates volume based on magnetic impedance. This machine is widely used in research and was developed specifically for measuring GCF and is easy to operate. Room temperature, humidity, sample evaporation, strip location within the machine and calibration of the machine are the important factors for assessment of GCF volume.[29] The Periotron device was placed in the clinic; the measurements were performed quickly and the device was recalibrated before each patient.

Overhanging dental restorations are associated with increased gingival inflammation and healing, increased marginal bone loss and deeper periodontal pockets.[30] Cytokines and growth factors secreted by macrophages are involved in the proliferation and migration of fibroblasts and endothelial cells into the wound area. Fibroblasts responsible for the replacement of the provisional extracellular matrix produce a new collagen-rich matrix approximately 1 week following wounding. In the early phase of inflammation, neutrophils and monocytes accumulate on the root surface and the late phase of inflammation dominate the healing picture as macrophages migrate into the wound within 3 days. Connective tissue attachment may be seen at the root surface at 7 days.[31,32] The presence of proinflammatory and inflammatory cytokines in GCF has been proposed as a potentially useful diagnostic or prognostic marker of periodontal destruction.[15,16,33] The changes of cytokine concentration in GCF were evaluated 7 days after overhang removal in this study.

Cytokine antibody array was used for determination of GCF cytokine level. Advantages of this array include a higher sensitivity and reduced variability relative to enzyme-linked immunosorbent assay (ELISA).[13]

The volume of GCF present at a given site may be directly related to tissue inflammation (including vascular permeability and the nature of the inflammatory infiltrate) as well as permeability and ulceration of the crevicular epithelium. Sites characterized as being moderately or severely inflamed exhibit a greater volume of GCF than less inflamed sites. Nevertheless, no studies have demonstrated that an increased volume of fluid in the crevice is associated with risk for periodontal tissue destruction.[13,34] Although the current study observed an increase in GCF volume after the renewal of overhang restorations, the difference was not significant. Moreover, GCF volume is influenced by many factors, such as flow rate, gingival trauma and repeat sampling.[35,36]

Mokeem[37] reported a significant decrease in probing depth, GI and GCF volume after removal of overhang amalgam restorations. Likewise, significant reductions in clinical parameters (PI, GI) were observed in the current study, showing improved periodontal health after the removal of the overhang amalgam restorations. Initiation and progression of gingival inflammation cannot be reliably assessed by measure-

ment of accumulated pro-inflammatory cytokines, because a specific pattern of synthesis and degradation of cytokines occurs within the gingiva, producing changes in the cytokine ratios as the disease progresses.[38] Excessive and/or continuous production of cytokines in periodontal tissues is known to be responsible for periodontal destruction. In particular, inflammatory cytokines, such as IL-1β, IL-6, IL-8 and TNF-α, are present in diseased periodontal tissues, and their unrestricted production results in chronic leukocyte recruitment and tissue destruction.[39] IL-8 is important for the initiation and development of inflammation and tissue destruction in periodontal diseases.[40] Erdemir et al[26] reported that non-surgical periodontal treatment reduced the total amount of IL-8, but not of IL-6, and the clinical parameters of teeth with fixed partial dentures (FPDs) and contralateral teeth. In contrast, Jin et al[41] showed lower concentrations of IL-8 in patients with periodontitis compared to healthy controls. Engebretson et al[42] showed no significant changes in total GCF TNF-α levels in patients with periodontitis after 3 weeks' periodontal therapy. These findings were similar to those of the present study in which IL-6, IL-8 and TNF-α exhibited no significant differences before and after restorative treatment. The differences between these studies may be attributed to several factors, such as patient and site selection, sampling method employed, timing of clinical reassessment, and possible variations in periodontal treatment protocol and treatment outcomes achieved.[26]

Paolantonio et al[4] compared the short-term clinical and microbiological features occurring in the gingiva after the completion of different subgingival restorations. They reported that no significant changes in the composition of the subgingival microflora were observed in amalgam. Similar results were observed in a previous study[43] that evaluated the antibacterial properties of three resin composites (Z250, Tetric Ceram and P60), and dental amalgam in vitro showing that amalgam completely inhibited the growth of the tested bacteria. No significant difference was determined between baseline GCF IL-6, IL-8 and TNF-α values and those measured 1 week later. This may be attributed to the short follow-up period and existing antibacterial effects of amalgam restorations. This activity has been related to the presence of mercury, copper and zinc.

In conclusion, the results of this study showed that there was no difference in the concentration of TNF-α, IL-6 and IL-8 in GCF before and after restorative treatment. However, there was a trend toward lower clinical parameters after restorative treatment. These observations further highlight the importance of removing the overhanging margins of restorations as an important component of initial periodontal therapy. Further longitudinal studies involving larger populations and long-term follow up are needed to confirm these findings and to better understand the mechanism of release of cytokines in overhanging restorations.

a. Dycal, Dentsply Caulk, Milford, DE, USA.
b. ProFlow Inc, Amityville, NY, USA.
c. Oraflow Inc, Plainview, NY, USA.
d. ISOLAB, Wertheim, Germany.
e. SPI Supplies Inc, West Chester, PA, USA.
f. Biosource, Europe SA, Nivelles, Belgium.
g. SPSS Inc., Chicago, IL, USA.

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