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Smoking cessation increases gingival blood flow and gingival crevicular fluid

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Abstract

Objectives: The purpose of the present study was to determine the effect of smoking cessation on gingival blood flow (GBF) and gingival crevicular fluid (GCF).

Material and Methods: Sixteen male smokers (aged 22–39 (25.3 ± 4.0) years), with no clinical signs of periodontal and systemic diseases, were recruited. The experiment was performed before (baseline) and at 1, 3 and 5 days, and at 1, 2, 4 and 8 weeks after smoking cessation. The status of smoking and smoking cessation was verified by exhaled carbon monoxide (CO) concentration, and by serum nicotine and cotinine concentrations. A laser Doppler flowmeter was used to record relative blood flow continuously, on three gingival sites of the left maxillary central incisor (mid-labial aspect of the gingival margin and bilateral interdental papillae). The GCF was collected at the mesio- and disto-labial aspects of the left maxillary central incisor and the volume was calculated by the Periotron 6000® system. The same measurements except for the GBF were performed on 11 non-smoking controls (four females and seven males), aged 23–27 (24.4 ± 1.2) years.

Results: Eleven of 16 smokers successfully completed smoking cessation for 8 weeks. At 1 day after smoking cessation, there was a significantly lower CO concentration than at baseline ($p<0.01$). Also, nicotine and cotinine concentrations markedly decreased at the second measurement. The GBF rate of smokers was significantly higher at 3 days after smoking cessation compared to the baseline ($p<0.01$). While the GCF volume was significantly increased at 5 days after smoking cessation compared to the baseline ($p<0.01$), it was significantly lower than that of non-smokers until 2 weeks after smoking cessation ($p<0.01$).

Conclusion: The results show that the gingival microcirculation recovers to normal in the early stages of smoking cessation, which could activate the gingival tissues metabolism/remodeling, and contribute to periodontal health.

Key words: gingival blood flow; gingival crevicular fluid; smoking cessation

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There are numerous reports that smoking affects periodontal tissue. Cigarette smoking is clearly established as one of the most significant risk factors in the development and the progression of periodontal disease (Bergström 1989, Bergström & Preber 1994, Haber 1994a). Several clinical and epidemiological studies indicate that cigarette smoking has harmful effects on the response to a variety of non-surgical (Preber & Bergström 1985) and surgical procedures including: modified Widman flap surgery (Preber & Bergström 1990), guided tissue regeneration (Tonetti 1995), dental implants (Lambert et al. 2000) and supportive periodontal treatment (Kaldahl et al. 1996). Also, Raulin et al. (1988) have reported that nicotine contaminated root surfaces affects fibroblast attachment to root surfaces and may interfere with healing and/or new attachment. Smoking also detrimentally affects the neutrophils and macrophages, which are important as gingival immunocompetent cells. Especially, smoking impairs neutrophils chemotaxis and/or phagocytosis (Eichel & Shahrik 1969), and stimulates or impairs the oxidative burst (Ryder et al. 1998).

Hence, quitting smoking is absolutely essential for improving the healing potential before beginning periodontal treatments (Kinane & Chestnutt 2000, Mullally 2002). It is reported that smoking cessation appears to return the periodontal healing response to the same level as in non-smokers, and also

that responses of former smokers are comparable with those of non-smokers (Kaldahl et al. 1996, Grossi et al. 1997). Haber & Kent (1992) also have suggested that the effects of smoking seemed reversible, because the risk of developing periodontitis was clearly reduced upon smoking cessation. These findings provide the evidence that smoking cessation is beneficial for periodontal therapy. Furthermore, the understanding that smoking is a significant risk factor for periodontal disease implies that smoking cessation is advantageous.

In comparing former smokers to current smokers, several epidemiological studies have revealed that: the prevalence and severity of periodontitis is less (Haber 1994b), the odds ratio in the presence of moderate or advanced periodontitis compared with never-smokers is lower (Haber & Kent 1992), the rates of tooth loss in men were reduced (Krall et al. 1997) and the progression of bone loss was retarded (Bolin et al. 1993). However, little information is available about the effects of cigarette smoking cessation on gingival health (Liede et al. 1999), and the process remains still under discussion: the relationship between the non-smoking period and gingival recovery is not discussed enough and is worth investigating.

The gingival blood flow (GBF) and the gingival crevicular fluid (GCF) are well-known markers of gingival health and have been used in many studies (Persson et al. 1999, Meekin et al. 2000). It has been reported that the GCF volume was lower in smokers than in non-smokers (Hedin et al. 1981, Persson et al. 1999). On the other hand, no direct comparison of the GBF between non-smokers and smokers has been reported yet, because of the assay-limit of the laser Doppler flowmeter (LDF). The unanswered question to date is how these markers change by smoking cessation. In the present study, we investigated the alteration of gingival microcirculation due to smoking cessation for 8 weeks, by measuring GBF level and GCF volume.

Material and Methods

Subjects

The study groups consisted of 16 smokers (test group) and 11 non-smokers (control group) who had no clinical signs of systemic diseases and periodontal diseases. Both of the groups were recruited from dental students of the Faculty of Dentistry, Niigata University, Niigata, Japan. The age of the smokers ranged from 22 to 39 years and the non-smokers from 23 to 27 years. Subjects who had less than 24 teeth, who had taken antibiotics, anti-inflammatory drugs or immunosuppressive drugs during the previous 8 weeks, or who had gone through periodontal therapy within the past 6 months, were excluded from the study. Subjects were selected on the basis of reported smoking habits, namely, smokers who claimed to have smoked at least 10 cigarettes per day for the past 5 years at the minimum. Non-smokers were defined as people who had never smoked. Group allocation was subsequently confirmed according to serum cotinine and exhaled carbon monoxide (CO) levels. Smokers were required to demonstrate a cotinine level of >18 ng/ml and a CO level of >8 ppm. Non-smokers were required to show a baseline cotinine level of <5 ng/ml and CO level of <7 ppm. Clinical registrations of gingival index (GI), probing pocket depth (PPD) and probing attachment level (PAL) were made 1 month prior to starting the study by one periodontist. With the WHO probe, the GI was scored at four sites per tooth (mesio-buccal, buccal, disto-buccal, lingual), and PPD and PAL were determined at six sites per tooth (mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual, disto-lingual). The demographic data for subjects is presented in Table 1.

Experimental protocol

In order to calculate the changes in GBF level and GCF volume over time by smoking cessation, smoker subjects were monitored over an 8-week period at the following times: before (baseline), at 1, 3 and 5 days, and at 1, 2, 4 and 8 weeks after smoking cessation. Subjects preserved their habit of everyday life during the period including ways of brushing and kinds of toothbrush and toothpaste. In the event of illness or health problems, subjects were withdrawn from the study. The measurements were performed between 16:00 and 18:00 hours, in a silent room with a temperature at 23–25°C. Subjects were instructed to keep absolutely quiet, forbidden to brush, gargle or eat and drink anything for 30 min prior to taking measurements. Also prior to measurements, a physical condition was checked to determine if they were in normal health. The mean arterial blood pressure and heart rate were recorded using a sphygmomanometer (Kentarou BP-203RV II®, COLIN Co., Aichi, Japan) attached to the left arm, and finally body temperature was taken by clinical thermometer (Electronic Thermometer®, TERUMO Co., Tokyo, Japan). Fifteen minutes before the following measurements, subjects were seated in an upright comfortable position in a dental chair. The study area was the gingiva around the left maxillary central incisor in both measurements. None of the teeth sampled had any defective restorations, crowns, calculus, caries or gingival bleeding, and all examinations were done by the same examiner. In the first experiment, GBF was measured by means of LDF. Second, sampling of GCF was carried out as described later. The investigation was approved by the local ethical committee of the Niigata University Graduate School of Medical and Dental Sciences, and all subjects signed informed consent to participate in the study.

Status of smoking

Status of smoking and smoking cessation was verified by CO concentration, by serum nicotine and cotinine concentrations in addition to self-reports. The peripheral blood was taken from all participants. For the quitters (test group) the blood was taken four times: before smoking cessation and after 1, 4 and 8 weeks. In the control group the blood was taken only once. The collection of venous blood samples from a forearm vein, avoiding the hand used for smoking, was performed between 11:00 and 11:30 hours, in consideration of the effect of blood flow (Russell et al. 1980). The

Table 1. Characteristics of the study population

	Male	Female	Age (years)	Pack year	GI	PPD (mm)	PAL (mm)
smoker	16	0	25.3 ± 4.0	9.5 ± 6.8	0 ± 0.1	1.9 ± 0.7	1.9 ± 0.7
non-smoker	7	4	24.4 ± 1.2		0 ± 0.1	1.9 ± 0.6	1.9 ± 0.6

GI, gingival index; PPD, probing pocket depth; PAL, probing attachment level.
Values are expressed as mean ± SD.

blood samples were left at room temperature for 1 h, then centrifuged at $2000 \times g$ for 20 min. Serum nicotine and cotinine levels were analysed by BML General Laboratory (Saitama, Japan). The Micro Smokerlyzer® (Bedfont Scientific Ltd, Kent, UK), which is an exhaled CO monitor used during smoking cessation programs to give the smoker visible proof of the damaging CO levels, was applied to the measurement of expired CO concentration before every experiment. In brief, subjects were asked to hold their breath for 15 s after a deep breath, then to slowly blow all of it through a cardboard tube into the machine's mouthpiece.

Laser Doppler flowmetry

A Moor DRT4 laser Doppler flowmeter® (Moor Instruments Ltd, Axminster, UK) was used to evaluate the relative blood flow of the gingiva. The methodology and operating principles of this equipment have been previously described by Nilsson et al. (1980) and Baab & Oberg (1987), and patterned from the works of Meekin et al. (2000). Until now, most reports have compared records of continuous readout of the average blood flow flux in the gingival tissues. Because it is a relative measure, it is difficult to compare recordings from one time point to another. However, we checked the repeatability using individual splints as described later, developed a method to compare recordings among discontinuous time points. The gingival areas selected for observation were three sites of the central incisor: the mid-labial aspect of the gingival margin and the bilateral interdental papillae. The measurements were taken twice at each site to ensure reproducibility, and the average value was adopted as the representative value.

A type DP13® (Moor Instruments Ltd) with a 1.5 mm external diameter and 0.2 mm optic fiber core diameter was used as the probe for measurement. Each probe carried two optical fibers, one for transmitting and one for collecting back-scattered light. The centers of the fibers in the gingival probe were 0.5 mm apart at the tip. For the probe to be more convenient to use in the mouth, the length of the terminal stainless-steel shaft used was 10 mm. The dental probe used in this measurement was placed in such a way that it would record the vessels within the interdental papilla from the labial to the palatal. The display rate was set at 20/s and the time constant at 0.1 s, and the cut-off frequency was 14.9 kHz. The light source was a 3 mW semi-conductor laser that emits near-infrared light at an unadjustable wavelength of 780 nm. To fix the gingival laser probe in position at the papilla to be studied, alginate impressions were taken of the upper jaws and individual acrylic splints were made on the dental plaster casts. The splints covered the incisal, labial and palatal areas of the left maxillary central incisor and were about 2 mm thick. Before the placement of the splints, the surface of the mucosa and the tooth was dried with a short air blast. The gingival probe was placed in the splint, which had a small hole for the laser probe in contact with the chosen central incisor sites.

The flowmeter was used to measure concentration, which indicates the number of moving red blood cells in the tissue samples, and the average speed of red blood cells moving in the tissue sample volume. From tissue estimates, a calculation of the flux was done, which is related to the product of average speed and concentration of moving red cells in the tissue sample's volume. The back-scattered laser light intensity was used to check the efficiency of light collection by the laser Doppler probes.

GCF collection

GCF sampling was performed as previously described (Nomura et al. 1998). In brief, supragingival plaque was removed from the sites to be sampled. The sites to be sampled for GCF were isolated with cotton rolls and dried with a gentle stream of air to prevent contamination by saliva. A sterile Perio-paper® strip (Harco Electronics, Winnipeg, MB, Canada) was gently inserted into the orifice of the gingival crevice until mild resistance was felt, and left in place for 60 s at the mesio- and disto-labial aspects of the left maxillary central incisor. Samples visually contaminated with saliva or blood were discarded and excluded from the sampled group. This procedure was consecutively repeated four times at both sites, and the sum total GCF volume consisting of eight papers from each subject was immediately measured using a Periotron 6000® (Harco Electronics). The Periotron 6000® was calibrated before sample collection with known volumes of physiologic saline. Based on the calibration data, a linear regression standard curve was prepared for determining GCF volume.

Statistical analysis

The data obtained from the experiments are presented as means and standard deviations (SD). Flux measurements of blood flow have no absolute value, and proportional changes from baseline in smokers were used for comparisons between the time intervals. Alterations between baseline and time intervals in smokers were evaluated by repeated measures analysis of variance (ANOVA) with post hoc contrasts. The values of smokers at time intervals were compared with those of non-smokers in GCF volume analysis using the Student's *t*-test.

Results

As participants in this smoking cessation program, 11 of the 16 smokers aged 22–39 years (25.9 ± 4.7), kept systemic and oral health, successfully quit smoking for 8 weeks, and five smokers dropped out during the course of the study.

CO, nicotine and cotinine concentrations

Fig. 1 shows the mean and SD for exhaled CO concentration. The CO concentration in smokers was statistically significantly low at 1 day after smoking cessation, compared to the baseline (1 day versus baseline, 3.42 ± 1.51 versus 31.08 ± 5.96, $p < 0.01$). The CO concentration in smokers was statistically significantly higher than that of non-smokers at baseline only. The mean and SD for serum nicotine and cotinine concentrations from peripheral blood in each period and that of non-smokers are described in Fig. 2. After 1 week, nicotine and cotinine concentrations decreased below 5 ng/ml (lower limit of quantitation) in some of the samples. Although statistical analysis was not performed since the numbers of detectable subjects differed in each period, there seems to be a decreasing tendency in both nicotine and cotinine concentrations after smoking cessation. In non-smoker samples, neither nicotine nor cotinine were detected. These data show the success of a smoking cessation program for the study period.

Relative GBF

The mean and SD for the individual changes from baseline in smokers are

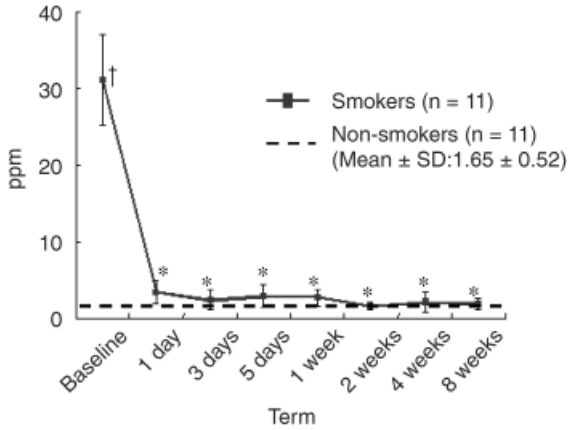

Fig. 1. Alterations of CO concentration (ppm) during 8-week non-smoking period (mean ± SD). *Significantly low values compared to the baseline in smokers ($p<0.01$). †Significantly high value compared to the non-smokers ($p<0.01$).

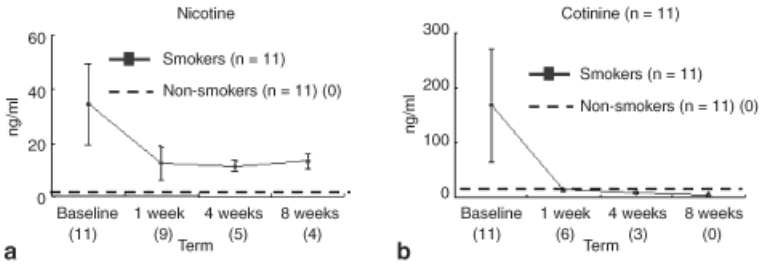

Fig. 2. Alterations of serum nicotine (a) and cotinine (b) concentrations (ng/ml) during 8-week non-smoking period (mean ± SD). The numbers in round brackets show the detectable subject of nicotine or cotinine.

shown in Table 2. The GBF rate was statistically significantly increased at 3 days after smoking cessation compared to the baseline ($p<0.01$).

The volume of GCF

The mean and SD for GCF volume of smokers at each period and that of non-smokers are shown in Table 3. The GCF volume was statistically significantly increased compared to the baseline at 5 days after smoking cessation ($p<0.01$). However, the GCF volume of smokers until 2 weeks after smoking cessation was statistically significantly low compared to non-smokers ($p<0.01$).

Discussion

The objective of the present study was to evaluate the alteration of gingival microcirculation due to smoking cessation, by measuring GBF level and GCF volume. We demonstrated that the GBF and the GCF increased quite quickly, suggesting that gingival microcirculation may recover fast following smoking cessation.

CO, nicotine and cotinine are known to be toxic constituents of cigarette smoke, and are also well-known as biological markers of exposure to cigarette smoking (Scott et al. 2001). We used these markers to estimate smoking status (levels of CO, nicotine and cotinine). Generally, CO generates CO–Hb by combining with hemoglobin, and diminishes the oxygen transport ability of blood. Tirlapur et al. (1983) reported that arterial oxygen saturation of hemoglobin was slightly lower in smokers than non-smokers. In addition, Hanioka et al. (2000a) reported that oxygen saturation of hemoglobin in healthy gingiva was significantly lower in smokers than in non-smokers. Nicotine is a major component of cigarette smoke, and is quickly converted to cotinine, its major metabolite. Nicotine absorption exerts several diverse systemic effects, causing blood pressure increase, palpitation of the heart and generalized vasoconstriction, due to a nicotine-dependent activation of the sympathetic nervous system. Also, nicotine has direct adverse effects on various functions of the cells in periodontal tissues (Giannopoulou et al. 1999).

CO monitoring is a convenient and physiological procedure (Jarvis et al. 1986). The standard values of non-smoker, light-smoker and heavy-smoker are 1–7, 8–19 and 20 ppm or more, respectively, according to the manufacture's protocol. These data are consistent with the data of Crowley et al. (1989). Levels of cotinine are generally reported to remain relatively constant in active smokers over long periods of time, and are reported to be in higher concentrations than nicotine. Nicotine has a serum half-life of 1–2 h, while a serum half-life of cotinine is approximately 20 h (Scott et al. 2001). On the other hand, CO half-life is 3 h (Scott et al. 2001). In this study, the CO concentration decreased at 1 day after smoking cessation compared to the baseline, and was comparable to that of non-smokers. Nicotine and cotinine concentrations markedly decreased at the second measurement. However, considering the half-life of nicotine and cotinine, they might be expected to decrease even at 5–6 days after smoking cessation. Jarvis et al. (1987) have verified the ability in several markers of smoking status, suggesting a reference level of serum cotinine concentration (<13.7 ng/ml) to differentiate non-smokers from smokers. Our data fulfilled the reference level. These results proved that quitting smoking was completed successfully in this study. These evaluations contributed not only to the monitoring of the non-smoking status, but also to sustaining the motivation of the subjects during the smoking cessation programs. Especially, CO monitoring was effective to help sustain motivation by charting the progress immediately. The CO, nicotine and cotinine levels were consistent with self-reports in the test group, both quitters and those who dropped out of the program.

Generalised vasoconstriction in the peripheral blood vessels due to sympathetic excitatory action of nicotine causes severe reduction in intra-arterial blood flow rates in the human body (Clarke et al. 1981, Powell 1998). Acute smoking caused an almost 30% reduction in LDF in the microcirculation of thumb skin (van Adrichem et al. 1992). There are conflicting results concerning the acute smoking effects on periodontium: Clarke et al. (1981) reported a decrease in GBF based on heat diffusion studies, while Baab & Oberg (1987) reported an increase in GBF using a

Table 2. Percentage changes in relative GBF during a non-smoking period

$n = 11$	Baseline	1 day	3 days	5 days	1 week	2 weeks	4 weeks	8 weeks
			*	*	*	*	*	*
mean	100	120.4	142.9	168.0	173.4	179.7	185.7	189.1
SD		8.4	12.7	33.6	31.3	29.8	31.6	29.9

*Significantly high compared to the baseline ($p < 0.01$).
GBF, gingival blood flow.

Table 3. Alterations for GCF volume during a non-smoking period (μl)

$n = 11$	Baseline	1 day	3 days	5 days	1 week	2 weeks	4 weeks	8 weeks	Non-smokers ($n = 11$)
	†	†	†	†*	†*	*	*	*	*
mean	0.48	0.69	0.81	0.97	1.09	1.25	1.38	1.46	1.48
SD	0.21	0.29	0.35	0.38	0.41	0.49	0.54	0.5	0.33

*Significantly high compared to the baseline ($p < 0.01$).
†Significantly low compared to the non-smokers ($p < 0.01$).
GCF, gingival crevicular fluid.

different methodology from Clarke et al. for smoke exposed tissues. Also, an increase of GCF volume due to the effect of cigarette smoking has been demonstrated by McLaughlin et al. (1993). It is known that chronic smoking may result in a decline of gingival microcirculation function; the recovery of LDF signals was significantly longer in smokers than in non-smokers (Ketabi & Hirsch 1997), the periodontal pocket oxygen tension is significantly lower in smokers than in non-smokers (Hanioka et al. 2000b). Although accumulating evidence of smoking effects is available, as far as we aware, no study has reported the effect of smoking cessation on GBF and/or GCF levels. Thus, this study is the first reporting the alteration of gingival microcirculation due to smoking cessation.

The GBF and GCF measurements showed a significant increase at 3–5 days after smoking cessation, and the volume of the GCF at 2 weeks after smoking cessation was similar to non-smokers. These findings suggest that gingival microcirculation could recover in the early stages of smoking cessation. The data may support the views that there is an early benefit of smoking cessation in terms of periodontal treatment outcome (Grossi et al. 1997), past smokers and non-smokers respond similarly to treatment (Kaldahl et al. 1996). Our results suggest that smoking cessation plays some important roles in the recovery from chronic smoking effects on the gingiva. Also, the reduction of pharmacological effects of nicotine on microcirculation may contribute to the gingival recovery process (Clarke et al. 1981). Furthermore, the change of blood vessel diameter (Meekin et al. 2000), oxygen saturation of hemoglobin in the gingival (Hanioka et al. 2000a), and levels of nutrition including vitamin C and E (Seri et al. 1999), could be associated with the long-term recovery of the gingival microcirculation system. Interestingly, our data showed that the GCF volume increased significantly following the significant increases in GBF. These phenomena may be explained by the fact that exudative and transudative fluid and plasma proteins arrive in the gingival crevice region after having left the vessels and travelled through the tissue to create the GCF (Egelberg 1967, Cimasoni 1983). Our data imply that a diminished peripheral blood flow leads to a diminished GCF flow. Therefore, we speculate that increased GBF and GCF act synergistically to promote periodontal health by improving gingival microcirculation.

In conclusion, the present investigation showed that the GBF and the GCF significantly increased within 5 days after smoking cessation. The study also found that GCF volume after 2 weeks of smoking cessation was comparable to that of non-smokers. We provide the first evidence concerning the recovery of the gingival microcirculation system resulting from smoking cessation. Our data suggest that the gingival microcirculation may recover in the early stages of smoking cessation, which could activate gingival tissue metabolism and local host immune responses. The findings support the view that smoking cessation is beneficial to prevention and treatment of periodontal disease.

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