J Clin Periodontol 2005; 32: 1208–1212 doi: 10.1111/j.1600-051X.2005.00854.x

Journal of Clinical Periodontology

Effect of smoking on the gingival capillary density: assessment of gingival capillary density with orthogonal polarization spectral imaging

J. A. Lindeboom[1,2], K. R. Mathura[1], S. Harkisoen[1], H. P. van den Akker[2] and C. Ince[1]

[1]Department of Physiology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; [2]Department of Oral and Maxillofacial Surgery, Academic Medical Center and Academic Center for Dentistry (ACTA), University of Amsterdam, Amsterdam, The Netherlands

Lindeboom JA, Mathura KR, Harkisoen S, van den Akker HP, Ince C: Effect of smoking on the gingival capillary density: assessment of gingival capillary density with orthogonal polarization spectral imaging. J Clin Periodontol 2005; 32: 1208–1212. doi: 10.1111/j.1600-051X.2005.00854.x. © Blackwell Munksgaard, 2005.

Abstract

Objectives: Microvascular changes because of smoking are frequently presumed in models because of the negative effect of smoking portrayed on the microcirculation. We hypothesized that cigarette smoke might lead to a decrease in gingival capillary density.

Materials and Methods: Capillary density was assessed with orthogonal polarization spectral (OPS) imaging, a technique using special optics by which a virtual light source is created at a depth of 1 mm within the mucosa. The light is absorbed by haemoglobin, resulting in an image of the capillaries in negative contrast. The gingival capillary density was measured in 20 healthy male dental students with a mean age of 25. Ten of the students were smokers and 10 were non-smokers. In each subject six images of the right maxillary pre-molar region were obtained, and the mean gingival capillary density was determined through the use of K&K software technology.

Results: The mean capillary density in smokers was 69.3 ± 8.9 capillaries per visual field compared with a mean capillary density in non-smokers of 60.6 ± 5.4 ($p = 0.33$).

Conclusion: No significant differences were found between the gingival capillary density of smokers and non-smokers.

Key words: capillary density; smoking; OPS imaging

Accepted for publication 1 September 2005

Smoking is often associated with many detrimental effects in several organ systems and has been directly related to medical problems in different specialties such as skin disease, pulmonary disease, cancer, macular degradation, and cardiovascular disease (Haber et al. 1993, Ryder 1996). Several studies have demonstrated deteriorating effects of smoking on oral tissues; however, the exact mechanisms behind the negative effect of smoking on oral disease processes as in periodontal diseases are not fully understood (Preber & Bergström 1985, Bergström et al. 1988). Tobacco smoke comes into direct contact with oral tissues and is responsible for delivering an array of chemical pollutants into the buccal cavity such as nicotine, carbon monoxide, and hydrogen cyanide. Smoking has a vasoconstrictive effect on the gingival microcirculation resulting in reduced blood flow in gingival tissues. It has been observed that significantly increased levels of TNF-α have been detected in gingival crevicular fluid in both current and former smokers who have received treatment or no treatment for periodontal disease (Boström et al. 1998a, b). In another study morphologic alteration in the microvasculature of the rat oral mucosa was observed after systemic administration of nicotine (Johnson et al. 1989). In animal studies, it has been suggested that cigarette smoke has more influence on the microcirculation than just nicotine alone (Chen et al. 2002). The effect of smoking on the human oral microcirculation however remains a matter of debate.

In the past, a variety of methods have been used for measuring microvascular gingival blood flow, but most were invasive. To date several non-invasive

technologies (capillaroscopy, laser Doppler flowmetry (LDF), and orthogonal polarization spectral (OPS) imaging) have been developed and used to observe the morphology, physiology, and pathophysiology of disease regarding in vivo microcirculation. Insufficient studies have led to more research involving new technologies to observe and analyse the microcirculation. LDF has been used to study the effects of smoking on gingival blood flow in humans. It was found that the relative gingival blood flow immediately rose during smoking and rapidly returned towards baseline within 10 min. (Baab & Öberg 1987). These results contradict findings in rabbit studies where the gingival circulation after an initial increase dropped below baseline levels (Clarke et al. 1981, Clarke & Sheperd 1984). It seems that the chronic effect of smoking on microcirculatory function can be anticipated in clinical and animal studies of the microvasculature. LDF is often used as it allows non-invasive measurements of blood flow in humans and allows total flow to be monitored through tissues. However, the flow of individual microvessels, the number of vessels with active flow and vessel diameter cannot be analysed swiftly using the laser Doppler approach.

OPS imaging was invented during the process of developing a videomicroscope to obtain high-contrast images of blood in the microcirculation using reflected light (Nadeau & Gromer 2000). The polarized light used to illuminate the area of interest is reflected by the background and absorbed by heamoglobin (Hb). Specific optical filtration allows the elimination of the light reflected at the surface of the tissue to produce high-contrast reflected light images of the microcirculation. The OPS imaging technique is particularly convenient for studying tissue protected by a thin epithelial layer, such as the oral mucosa. As OPS imaging's original description, several clinical studies have demonstrated its clinical use in humans (Groner et al. 1999, Mathura et al. 2001a, c, De Backer et al. 2002, Spronk et al. 2002, Pennings et al. 2004, Lindeboom et al. 2005). Microcirculatory changes are pronounced in smoking individuals, and we hypothesize that cigarette smoke might lead to a decrease in gingival capillary density. In this study, we assessed the capillary density of 10 healthy smoking and non-smoking subjects.

Material and Methods

For this study, 20 healthy male students were recruited from the dental faculty of the Academic Center for Dentistry Amsterdam (ACTA) of the University of Amsterdam. The 20 subjects were separated into two groups: 10 smokers and 10 non-smokers. All smokers had been habitually consuming 15–25 cigarettes a day for at least 5 years. Women were purposely excluded to avoid potential hormone-induced microcirculatory changes. The mean age of the smokers was 25 ± 1.2 years with a comparable age distribution in the non-smoking group of 25 ± 1.4 years. The mean and standard deviations (SD) were calculated for smokers according to the amount of cigarette packs smoked in years (5.5 ± 2.7 years). Smoking subjects were selected on the basis of their reported smoking habits, namely smokers who have claimed to have smoked at least 10 cigarettes/day for the last 5 years. Non-smokers were defined as people who had never smoked. Subjects were all healthy and prior to inclusion in the study, any type of gingivitis or periodontitis was ruled out. Exclusion criteria included reports of their personal histories regarding hypertension, angina, any inflammatory disease (rheumatoid arthritis or eczema), the use of antibiotics, anti-inflammatory or immunosuppressive drugs. At intake, no differences were found between smokers and non-smokers with regard to gingival bleeding, plaque index and dental hygiene in general (Table 1). Plaque deposits were scored using the plaque index score (Silness & Löe 1964), and gingival conditions were scored using the gingival index score system (Löe & Silness 1963).

The purpose of the study was explained to the participants, and the study was approved by the Academic Medical Center's Institutional Medical Ethics Committee. A signed informed consent was obtained from all participants.

Table 1. Plaque and bleeding scores in the smoking and non-smoking dental students

	Mean plaque index (SD)	Mean bleeding index (SD)
Smokers	0.47 (0.14)	0.08 (0.08)
Non-smokers	0.43 (0.13)	0.07 (0.07)
p-value	0.72	0.6

Data are expressed as mean (SD).

OPS measurements

OPS technology was incorporated into a small portable handheld device, and the capillary density and the microvascular network of the gingival mucosa were studied with a $\times 5$ objective providing a $\times 325$ magnification. No measurements were made within 30 min. of the subject's last food, drink or cigarette intake. In addition, none of the subjects had brushed, flossed or rinsed their teeth in the preceding 30 min. prior to the measurements, to prevent any possible mechanical or thermal influences on the microvessel density. The subjects were seated in an upright dental chair in a room with a temperature of approximately 22°C. The OPS objective was covered with a sterile plastic cap and the device was applied to the buccal aspect of the gingiva of the first right maxillary premolar for measurements of the gingival capillary density. The marginal gingiva was chosen for measurements because of the standardization of the location and the ease of access for the OPS probe. Two minutes of images were recorded on a digital videotape. At offline analysis images were captured in intervals of every 20 s. Six images were used for quantification of the mean capillary density.

Vessel enumeration

The clinical data were analysed by counting the capillary density/visual field/images quantitatively using K&K software (Nolte et al. 1995). Quantitative analysis of functional capillary density was performed by means of a computer-assisted video analysis system, which allows calculation of the capillaries. Using the superimposition of a virtual grid system the number of vessels could be determined. The images were randomly quantified by presenting them in a random order.

Statistical analysis

The mean age and capillary density comparison between the two groups were performed using an independent *t*-test. Sample size was calculated on the basis of earlier publications (Bergström et al. 1988, Persson & Bergström 1998). A sample size of 10 in each group would have an 80% power to detect a difference of 10–15 vessels per visual field using a two group *t*-test with a 0.05 two-sided significance level. Calculations

were performed using the SPSS software program (SPSS 12.0, SPSS Inc., Chicago, IL, USA).

Results

The mean capillary density in smokers was 69.3 ± 8.9 capillaries per visual field compared with a mean capillary density in non-smokers of 60.6 ± 5.4. Figure 1 shows an OPS image of a non-smoking subject compared with an OPS image of a smoking subject. Figure 2 illustrates a Whisker plot showing the comparison between mean capillary density ranges of non-smokers and smokers. The mean capillary density is higher in the smoking group as is the SD. There is, however, no statistically significant difference ($p = 0.33$).

Discussion

The present study is unique because it attempts to use the OPS imaging technology to analyse and measure capillary densities in normal healthy human gingiva in both smokers and non-smokers. In light of the debate regarding whether oral microcirculatory irregularities could be directly associated with tobacco smoke, this study aimed at assessing whether differences were present in gingival capillary density in the gingiva particularly with a decrease in gingival capillary density in smokers. The present study did not deal with the direct effects of cigarette smoke on the gingival microcirculation as those may be transient. Our question was rather as to whether smoking caused chronic microcirculatory deficiency, resulting in decreased gingival capillary density.

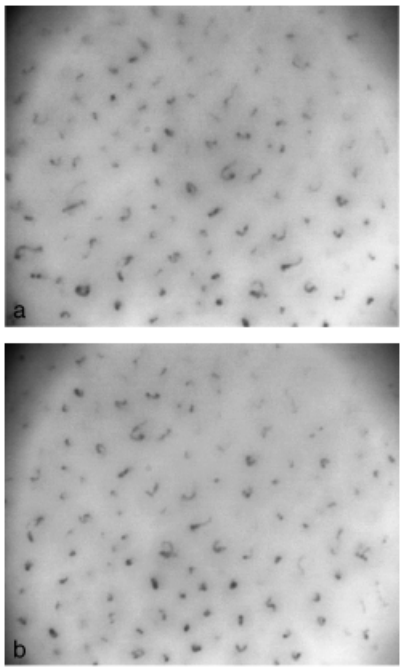

Fig. 1. Orthogonal polarization spectral (OPS) image of a smoking subject (a) compared with a non-smoking subject (b). The gingival capillary loops are nicely visible.

Several imaging methods have been used to study human gingival capillary and the effects of smoking on these microvascular structures. These methods include laser Doppler (Baab & Öberg 1987), stereophotography (Persson & Bergström 1998), tissue reflectance spectrophotometry (Hanioka et al. 2000) and videomicroscopy (Scardina & Messina 2004). Table 2 summarizes these techniques. Most techniques to study the microcirculation however are not applicable for proper assessment of the gingival microcirculation in humans because of the use of contrast, their invasiveness, the size of the instruments and probes and the lack of reproducibility of data. OPS images obtained in this study were of good quality compared with other image qualities described elsewhere by capillaroscopy (Mathura et al. 2001b).

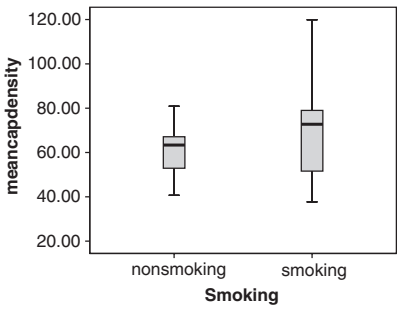

Fig. 2. Mean gingival capillary density in smokers and nonsmokers. No statistically significant difference was found ($p = 0.33$).

No significant differences in gingival capillary density were observed between the smokers and non-smokers. This was not unexpected because subjects were young healthy males as determined by health history and initial screening. The distribution of age among subjects in this study was normal, with no significant difference in the mean age between the smokers and non-smokers. All recruited subjects were male, to avoid confounding influences on the vascular density due to hormonal factors. Although one might postulate a significant difference in contrast to our hypothesis, no statistically differences in capillary densities were found in other studies (Persson & Bergström 1998, Mirbod et al. 2001). Persson & Bergström (1998) studied the vascular density of the healthy marginal gingiva in seven smoking and seven non-smoking female dental students using low-power stereophotography. The mean age of the subjects was 30.4 years, which was higher than the age of subjects selected in this study. A standardized region for measurements was chosen in the present study to assess the buccal marginal gingiva, using the first maxillary premolar as the reference measurement location. The region in the oral cavity that was selected for this investigation differs from other approaches, which used the maxillary anterior region in nine of the subjects and the mandibular anterior region in five of the subjects (Persson & Bergström 1998). Another study applied immunohistochemical staining of vestibular gingival biopsies to assess the effect of smoking on the gingival vascular density (Mirbod et al. 2001). As in our study, they failed to find significant differences in vascular densities between smokers and non-smokers; however, the findings revealed that there was a higher percentage of smaller blood vessels and a lower percentage of larger vessels in smokers. In another investigation, higher numbers of capillaries in the labial mucosa existed in smokers when compared with non-

Table 2. Techniques applied to assess the effects of smoking on the microcirculation

Baab & Öberg (1987)	Human subjects	Laser Doppler	The theory that smoking impairs gingival blood flow may not be true in humans
Persson & Bergström (1988)	Human subjects	Stereophotography	The gingival vascular density is uninfluenced by smoking
Hanioka et al. (2000)	Human subjects	Tissue reflectance spectrophotometry	Smokers have functional impairments in gingival microcirculation
Scardina & Messina (2004)	Human subjects	Videomicroscopy	Smokers have a higher detectable number of capillaries

smokers (Scardina & Messina 2004). The results of that study might not be comparable with the present study because measurements were taken from the labial mucosa and not the gingival mucosa. Other studies on gingival blood flow also yield contradictory findings. Through the use of LDF, no differences between smokers and non-smokers and their gingival blood flow were found (Meekin et al. 2000). Other studies reported a significant increase in gingival blood flow during smoking despite the considerable variation between subjects (Baab & Öberg 1987, McLaughlin et al. 1993).

The exact mechanism of negative effects expressed on the oral mucosa is not exactly known. Studies have shown detrimental effects of smoking on the periodontium, resulting in a greater loss of clinical attachment and alveolar bone and an increased number of deep pockets than in non-smokers (Bergström et al. 1986, Brochut & Cimasoni 1997). Many host systems, both local and systemic, appear to be affected. Locally, smoking causes a reduction in the gingival blood flow with a decreased number of circulating cells and less oxygen transport to the gingiva, thus weakening its integrity and its defensive reparative posture (Bergström & Preber 1986, Bergström et al. 1988). It has been reported that there is a lower function of oxygen sufficiency in healthy gingiva in smokers compared with non-smokers as well as evidence that smokers have impaired oxygen function in both healthy and inflamed gingival (Hanioka et al. 2000). Interestingly, another study investigated the gingival blood flow after administration of a local anaesthetic containing adrenaline and compared 20 smokers with 20 non-smokers using LDF (Ketabi & Hirsch 1997). The injection of local anaesthetics containing adrenaline resulted in a similar drop of LDF signals in both the smoker and non-smoker groups but the recovery of LDF signals to baseline took significantly longer in smokers than in non-smokers. This was either because of reduced metabolism of adrenaline in smoker's gingival tissue or nicotine stimulated release of catecholamines, particularly adrenaline. Endogenous adrenaline released in response to smoking may have added to the effects of adrenaline administered in local anaesthetics and may have further prolonged the period of reduced LDF values. In previous studies, the vascular reaction of the marginal gingiva in smokers was less pronounced than non-smokers, confirming that gingival inflammation is suppressed in smokers (Bergström & Preber 1986).

In conclusion, the present study reports the first clinical use of OPS imaging to assess the gingival microcirculation. The findings of this investigation suggest that no differences in capillary densities exist between smokers and non-smokers. It is unlikely that differences in susceptibility to periodontal disease between non-smokers and smokers are due to differences in capillary density. Vascular differences as a result of smoking are more likely caused by the effects of smoking on capillary diameter and capillary flow in combination with effects of the immunomodulatory response in the host.

References

Baab, D. A. & Öberg, P. A. (1987) The effect of cigarette smoking on gingival blood flow in humans. *Journal of Clinical Periodontology* **14**, 418–424.

Bergström, J., Persson, L. & Preber, H. (1988) Influence of cigarette smoking on vascular reaction during experimental gingivitis. *Scandinavian Journal of Dental Research* **96**, 34–39.

Bergström, J. & Preber, H. (1986) The influence of cigarette smoking on the development of experimental gingivitis. *Journal of Periodontal Research* **21**, 668–676.

Boström, L., Linder, L. E. & Bergström, J. (1998a) Influence of smoking on the outcome of periodontal surgery. A 5-year follow-up. *Journal of Clinical Periodontology* **25**, 194–201.

Boström, L., Linder, L. E. & Bergström, J. (1998b) Clinical expression of TNF-α in smoking associated periodontal disease. *Journal of Clinical Periodontology* **25**, 767–773.

Brochut, P. F. & Cimasoni, G. (1997) Tabagisme et parodonte. I. Historique et aspects cliniques. *Revue Mensuelle Suisse d'Odontostomatolgie* **107**, 673–686.

Chen, S. H., Lee, Y., Wei, F., Huang, J. & Chen, H. (2002) In vivo evaluation of leucocyte dynamics in cremaster muscle in rats after exposure to cigarette smoke. *Scandinavian Journal of Plastic Reconstructive Surgery and Hand Surgery* **36**, 321–325.

Clarke, N. G. & Sheperd, B. C. (1984) The effects of epinephrine and nicotine on gingival blood flow in the rabbit. *Archives of Oral Biology* **29**, 789–793.

Clarke, N. G., Sheperd, B. C. & Hirsch, R. S. (1981) The effects of intra-arterial epinephrine and nicotine on gingival circulation. *Oral Surgery Oral Medicine Oral Pathology* **52**, 577–582.

De Backer, D., Creteur, J., Preiser, J. C., Dubois, M. J. & Vincent, J. L. (2002) Microvascular blood flow is altered in patients with sepsis. *American Journal of Respiratory and Critical Care Medicine* **166**, 98–104.

Groner, W., Winkelman, J. W., Harris, A. G., Ince, C., Bouma, G. J., Messmer, K. & Nadeau, R. G. (1999) Orthogonal polarization spectral imaging: a new method for study of the microcirculation. *Nature Medicine* **5**, 1209–1212.

Haber, J., Wattles, J., Crowley, M., Mandell, R., Joshipura, K. & Kent, R. L. (1993) Evidence for cigarette smoking as a major risk factor for periodontitis. *Journal of Periodontology* **64**, 16–23.

Hanioka, T., Tanaka, M., Ojima, M., Takaya, K., Matsumori, Y. & Shizukuishi, S. (2000) Oxygen sufficiency in the gingiva of smokers and non-smokers. *Journal of Periodontology* **71**, 1846–1850.

Johnson, G. K., Fung, Y. K. & Squier, C. A. (1989) Effects of systemic administration of nicotine on capillaries in rat oral mucosa. *Journal of Oral Pathology and Medicine* **18**, 230–232.

Ketabi, M. & Hirsch, R. S. (1997) The effect of local anesthetic containing adrenaline on gingival blood flow in smokers and non-smokers. *Journal of Clinical Periodontology* **24**, 888–892.

Lindeboom, J. A., Mathura, K. R. & Ince, C. (2005) The microcirculation during wound healing after oral and maxillofacial surgical procedures. In: Vincent, J. L. (ed.). *Yearbook of Intensive Care and Emergency Medicine*, pp. 519–527. Berlin Heidelberg: Springer-Verlag.

Löe, H. & Silness, J. (1963) Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontologica Scandinavica* **21**, 533–551.

Mathura, K. R., Alic, L. & Ince, C. (2001a) Initial clinical experience with OPS imaging. In: Vincent, J. L. (ed.). *Yearbook of Intensive Care and Emergency Medicine*, pp. 233–244. Berlin Heidelberg: Springer-Verlag.

Mathura, K. R., Bouma, G. J. & Ince, C. (2001c) Abnormal microcirculation in brain tumors during surgery. *The Lancet* **17**, 1698–1699.

Mathura, K. R., Vollebregt, K. C., Boer, K., de Graaf, J. C., Ubbink, D. T. & Ince, C. (2001b) Comparison of OPS imaging and conventional capillaroscopy to study human microcirculation. *Journal of Applied Physiology* **91**, 74–78.

McLaughlin, W. S., Lovat, F. M., Macgregor, I. D. & Kelly, P. J. (1993) The immediate effects of smoking on gingival fluid flow. *Journal of Clinical Periodontology* **20**, 448–451.

Meekin, T. N., Wilson, R. F., Scott, D. A., Ide, M. & Palmer, R. M. (2000) Laser Doppler flowmetry measurements of relative gingival and forehead skin blood flow in light and heavy smokers during and after smoking. *Journal of Clinical Periodontology* **27**, 236–242.

Mirbod, S. M., Ahing, S. I. & Pruthi, V. K. (2001) Immunohistochemical study of vestibular gingival blood vessel density and internal circumference in smokers and non-

smokers. *Journal of Periodontology* **10**, 1318–1323.

Nadeau, R. G. & Gromer, W. (2000) Orthogonal Polarization Spectral Imaging: state of the Art. In: Messmer, K. (ed.). *OPS-Imaging – A New Tool for Microcirculation Research*, pp. 9–20. Basel: Karger.

Nolte, D., Zeintl, H., Steinbauer, M., Pickelmann, S. & Messmer, K. (1995) Functional capillary density: an indicator of tissue perfusion? *International Journal of Microcirculation and Clinical Experiments* **15**, 244–249.

Pennings, F. A., Bouma, G. J. & Ince, C. (2004) Direct observation of the human cerebral microcirculation during aneurysm surgery reveals increased arteriolar contractility. *Stroke* **35**, 1284–1288.

Persson, L. & Bergström, J. (1998) Smoking and vascular density of healthy marginal gingiva. *European Journal of Oral Sciences* **106**, 953–957.

Preber, H. & Bergström, J. (1985) Occurrence of gingival bleeding in smoker and non-smoker patients. *Acta Odontologica Scandinavica* **43**, 315–20.

Ryder, M. I. (1996) Position paper. Tobacco use and the periodontal patient. *Journal of Periodontology* **67**, 51–56.

Scardina, G. A. & Messina, P. (2004) Smoking habit and labial microcirculation. *Italian Journal of Anatomy and Embryology* **109**, 95–103.

Silness, J. & Löe, H. (1964) Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontologica Scandinavica* **22**, 121–135.

Spronk, P. E., Ince, C., Gardien, M. J., Mathura, K. R., Oudemans-van Straaten, H. M. & Zandstra, D. F. (2002) Nitroglycerin in septic shock after intravascular volume resuscitation. *The Lancet* **360**, 1395–1396.

Address:
J. A. H. Lindeboom
Department of Oral and Maxillofacial Surgery
Academic Medical Center
University of Amsterdam
Meibergdreef 9,
1105 AZ Amsterdam
The Netherlands
E-mail: j.a.lindeboom@amc.uva.nl