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Morphologic changes in the microcirculation induced by chronic smoking habit: A videocapillaroscopic study on the human gingival mucosa

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ABSTRACT: ***Purpose:*** To analyze the gingival characteristics of microcirculation *in vivo* and to evaluate the effects of smoking by means of computerised videocapillaroscopic techniques. ***Methods:*** 35 healthy non-smokers and 35 healthy smokers were examined. The characteristics of gingival microcirculation were examined using computerized video-microscopic techniques. For each subject, visibility, course, tortuosity and any images characteristic of capillary loops, besides the possible presence of microhemorrhages, the average caliber of the capillary loops and the number of capillary loops visible per square millimeter were evaluated. The investigation was non-invasive and repeatable for each subject. ***Results:*** The investigation of gingival mucosa revealed capillary loops with a course both parallel and perpendicular to the surface: close to marginal gingiva, the tops of the capillary loops often appeared as evenly distributed dots or commas; close to the papilla, microcirculation more frequently ran parallel to the surface, and its characteristics were similar to nail microcirculation. In smokers, it was possible to underline capillaries of a smaller caliber ($P < 0.001$) and a higher number of detectable capillaries ($P < 0.001$). An important correlation between the capillary tortuosity, close to the papilla, and the smoking habit ($P < 0.001$) was observed. This study has shown that the smoking habit represents a real risk factor for periodontal disease, which greatly affects microcirculation. (*Am J Dent* 2005;18:301-304).

CLINICAL SIGNIFICANCE: This study has pointed out that capillaroscopy is a reliable method to study oral microcirculation *in vivo*, and showed that the chronic smoking habit induces significant changes in gingival capillary morphology, caliber and number, which means that such a habit plays a crucial role as a great risk factor in the etiopathogenesis of periodontal diseases.

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Introduction

Capillaroscopy is a non-invasive diagnostic technique fundamental to view the peripheral circulation and to study microangiopathies which are the signs of numerous pathologies, both in the diagnostic and in the monitoring phase of the disease.¹

The alterations in the capillaroscopic picture can represent, in fact, the only documentary evidence of an incipient disease. Compared to other pathologies, in which the microangiopathies exclusively represent a complication (diabetes mellitus), among collagenopathies, microcirculation alterations have been documented by histological examinations in the early stages of such a disease, even in clinically healthy areas.

Microangiopathy evaluation can be of valuable help in giving prognostic indications within a therapeutic intervention. Since capillaroscopy is non-invasive, not expensive, easily repeatable and comparable even after years, it permits the monitoring in time of any disease compromising microcirculation.²

The study of capillaries goes back to the distant past, even if the investigation methods did not permit close studies; only recently, it has been possible to investigate microcirculation in depth with greater and greater diagnostic possibilities. At first, the capillaroscopic investigation was carried out with a reflecting microscope connected to a camera. Such a technique was invalidated both by the fact that the observation was limited to one or at most two operators, and that, being an *in vivo* study, the image was never completely still, which made the photographic documentation difficult. Today, the optical probe videomicroscope permits the investigation under uniform

lighting at variable magnifications, viewing the capillaries directly on a monitor.³⁻⁷

Studies using capillaroscopy have provided various data concerning position, form, capillary caliber and architectural framework.^{4,8}

The smoking habit is an important risk factor for oral diseases. Many studies tried to analyse the masticatory mucosa microcirculation and the related smoking effects.⁹⁻¹¹ To our knowledge, no studies have made use of an original, simple, immediate, non-invasive and low-cost technique like video-capillaroscopy.

This study evaluated the gingival microcirculation characteristics and the related effects of smoking *in vivo*.

Materials and Methods

70 subjects - 35 smokers (17 females, 18 males; mean \pm SD age: 50.86 ± 11.94 years; range: 26-63 years) and 35 non-smokers (17 females, 18 males; mean \pm SD age: 50 ± 11.60 years; range: 25-62 years) were examined in our laboratory. Of the subjects, 35 had been smokers for at least 7 years, smoking an average of 18 to 20 cigarettes per day. The non-smokers had never smoked.

The subjects were included in the study if the accurate exam of their medical history and the objective examination of their oral mucosa showed that they were healthy.

Loe & Silness gingival index, as well as plaque index, was = 0 in the study area in all subjects. All the subjects gave their informed consent for the processing and use of their personal medical data in scientific papers, according to Italian law.

Subjects were examined by computerized videomicroscopic

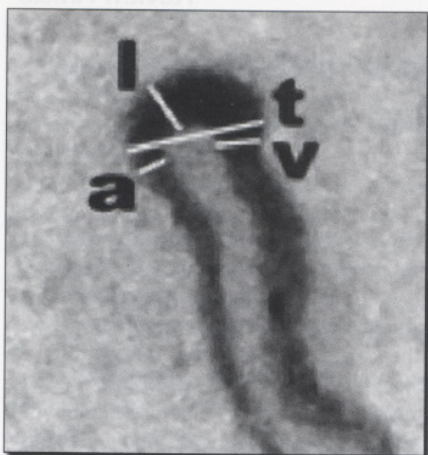


Fig. 1. The measurement execution mode: a: arteriolar; v: venule; t: caliber of the capillary loop (mag. x400).

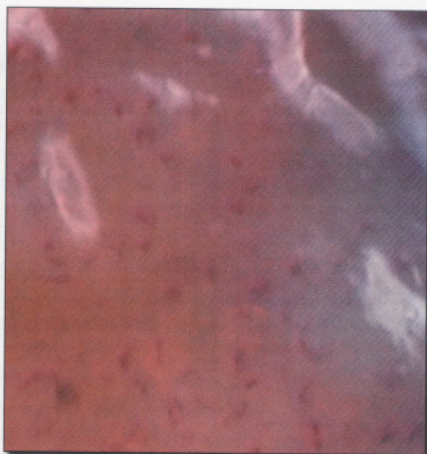


Fig. 2. Marginal gingiva: often (in 90% of the cases) it is possible to visualise only the apex of the capillaries, that appear as regularly distributed dots or comma like forms (mag. x200).

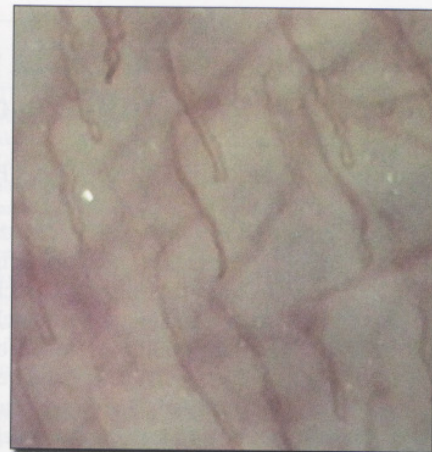


Fig. 3. Papilla: network of capillaries in polygonal mesh and a parallel orientation (type A) with respect to the surface in no-smoker subject (mag. x200).

techniques and related software (Videocap 100[®]).^{5,12,13} The optical probe videomicroscope is composed of a main unit, to which an optical probe with video-optical terminal is connected, and by a high resolution color monitor to view the examined area. The main unit is made of a cold halogen light source emitted by a 100W lamp provided with an electronic device which controls its light intensity, and a processing unit for the high definition video signal (420,000 pixels) provided with a color calibration device. The probe is equipped with a video-optical terminal containing a high definition video sensor, on which different variable magnification optics from x10 to x1000 can be applied. A technological characteristic of the video-optical terminal is the possibility to focus directly from the handpiece.

Image digitalization allows the analysis of the fundamental parameters of microcirculation (caliber and vessel length), and the calculation of the number of capillaries per mm² of the mucosa examined.

The capillaroscopic investigation was carried out with the patients in a sitting position, with the same light source, at the same room temperature (23°C), in the morning, by the same operator and repeated twice for each examined area. The examined areas for each patient were the labial gingiva, at the lower incisors.

The examined area was always the same for each patient: - the interdental papilla corresponding to 3.1 and 4.1, and the gingival margin of 3.1.

Two independent observers examined all the images. The intraobserver and interobserver variability was assessed with the two observers evaluating the same randomly selected images twice.

The following static parameters were used:

Nonparametric data - Capillary loop visibility, scored from 1 to 4: (1) simple focusing - within 30 seconds from the beginning of the examination; (2) average focusing - over 30 seconds and within 2 minutes; (3) difficult focusing - over 2 minutes; (4) impossible focusing; orientation as regards the surface, scored A, B or AB: (A) capillary loop course parallel to the surface; (B) capillary loop course perpendicular to the surface; (AB) both parallel and perpendicular; capillary tortuosity scored from 0 to 3: (0) absence

of crossing in the capillary loops; (1) presence of crossing; (2) greater presence of crossing; (3) complete distortion of the capillary loops; micro-hemorrhages, scored 0 or 1: (0) absence; (1) presence; characteristics of the capillary loops, scored 0 or 1: (0) absence; (1) presence.

Parametric data: number of visible capillary loops in every square millimetre (value obtained from the average of the two observations for each examined area); capillary loop caliber (values obtained from the average of the two observations for each examined area).

Regarding the parametric data, it must be underlined that they originate from the software connected to the videocapillaroscope. The system is properly calibrated: to every optical magnification corresponds an exact metric pixel value in the digitalized image; therefore, the capillary caliber can be measured with considerable precision. Fig. 1 highlights the measurement execution mode.

All the patients involved in the study kept a dietary diary for a period of 3 months, in order to evaluate any dietary differences which might affect the capillary pattern observed.

The statistical significance of the differences between the smokers and the non-smokers was checked with the Student's *t*-test for independent samples with regard to parametric data, and with the Mann-Whitney test (MW test) with regard to nonparametric data. The level of significance was set to $P < 0.05$. Data analysis was carried out with StatView 5.0.1.^b The results obtained from each examined area represent the average values of the two observations.

Results

Intrasubject variability satisfied the hypothesis *a priori* of limited dispersion. For the parametric data, variability ranged between +2 and -2% with respect to the average value. For the nonparametric data, one score difference was observed at most.

No significant differences were detected between smokers and non-smokers in age (MW test).

Microcirculation visibility was fairly difficult in 52 subjects, difficult in 18 subjects.

Microcirculation architecture in marginal gingiva did not show a constantly parallel orientation of the capillary loops in

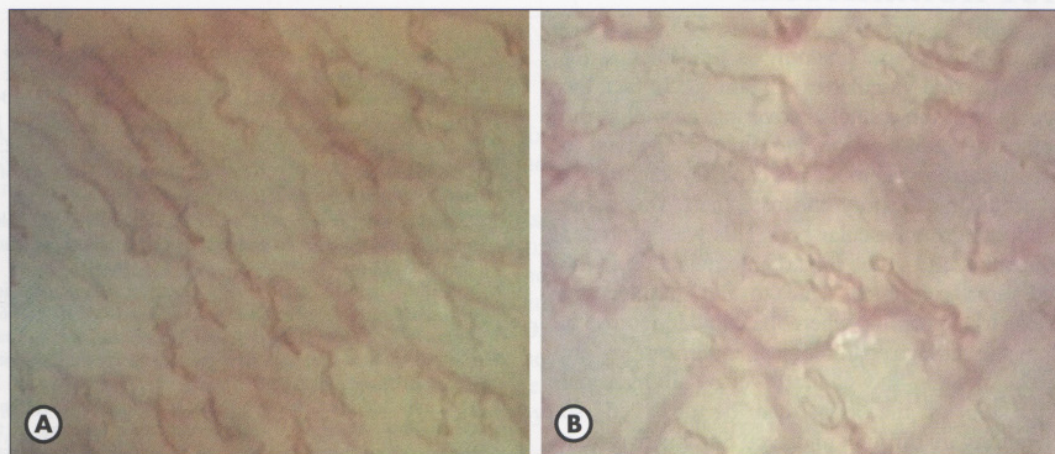


Fig. 4. A,B. papilla: capillaroscopic pattern in smoker subject (mag. x200).

relation to the surface, as did the fingernail bed. In 90% of the cases, only the apexes of the capillaries were visible; they appeared like evenly distributed dots or commas, resulting in a loop course perpendicular to the surface (type B) (Fig. 2). Close to the papilla, a loop course parallel to the surface (type A) could be observed (80% of the cases), which led to a complete evaluation of the capillary loops, since not only their apex, but also their entire course was visible (Fig. 3).

Capillary tortuosity obtained a score equal to 0 in all the cases of marginal gingiva observed, both in smokers and in non-smokers. Close to the papilla, in non-smokers, the score was: 0 in 28 subjects, one in five subjects, two in two subjects; in smokers, the score was: 0 in four subjects, one in 10 subjects, two in 16 subjects, three in five subjects ($P < 0.001$) (Figs. 4 A,B). Microhemorrhages were detectable in 31% of the smokers (Score 1).

The capillary loops showed variable diameters, courses and lengths; next to those with a horse stirrup shape, there were capillary loops similar to hairpins or commas.

The caliber of visible capillary loops was $8.63 \pm 1.08 \mu\text{m}$ (mean \pm SD) close to the papilla in non-smokers, and $6.31 \pm 1.58 \mu\text{m}$ (mean \pm SD) in smokers ($P < 0.001$).

The number of visible capillaries was 14.45 ± 4.10 (mean \pm SD) in the area of marginal gingiva and 8.63 ± 1.08 (mean \pm SD) close to the papilla in non-smokers; in smokers, it was 18.6 ± 4.67 (mean \pm SD) in the area of marginal gingiva and 12.77 ± 1.37 (mean \pm SD) close to the papilla ($P < 0.001$). No significant differences in the dietary habits of the subjects were detected. The results of the observations are summarized in the Table.

Discussion

The morphological-functional study of microcirculation is of fundamental importance; in fact, the microvascular bed is directly involved both in autoimmune etiopathogenesis pathologies, and in acute and chronic inflammatory etiopathogenesis pathologies.^{5,6,12,13}

Capillaroscopy is a very stimulating method for studying microcirculation, because of the possibility of studying small vessels *in vivo* by means of a microscope. Today, it is becoming more reliable thanks to the improvement of the observation tools (photography, videomicroscopy).^{1,2}

The value of capillaroscopic investigation as a diagnostic

Table. The characteristics of the gingival microcirculation in healthy smoker and no-smoker subjects. SD: Standard Deviation; ¹Differences between no-smoker and smoker subjects were tested by Mann-Whitney *U* test; S significant: $P < 0.001$.

	No smoker patients		Smoker patients	
	Score	Subjects	Score	Subjects
Orientation with respect to the surface close to the gingiva	B	90%	B	85%
	AB	8%	AB	10%
	A	2%	A	5%
Orientation with respect to the surface close to the papilla	A	80%	A	79%
	AB	12%	AB	21%
	B	8%		
Capillary tortuosity close to the gingiva	0	100%	0	100%
Capillary tortuosity close to the papilla	0	28%	0	4%
	1	5%	1	10%
	2	2%	2	16%
			3	5%
Microhemorrhages	0	100%	0	69%
			1	31%
Caliber of the capillary loops close to the papilla (mean \pm SD), Significance ¹ =S	$10.37 \pm 1.76 \mu\text{m}$		$6.31 \pm 1.58 \mu\text{m}$	
N°/mm ² close to the gingiva (mean \pm SD) Significance ¹ =S	14.45 ± 4.10		18.6 ± 4.67	
N°/mm ² close to the papilla (mean \pm SD) Significance ¹ =S	8.63 ± 1.08		12.77 ± 1.37	

means for peripheral microcirculation damage is confirmed by numerous studies. With the use of such a method, Halfoun¹⁴ showed that diabetic patients have capillary flow regulation abnormalities; with the same investigation technique, Haak¹⁵ showed that, in diabetic patients, the nervous reflex arcs are impaired. It must be said, however, that other studies^{1,2,12,16,17} have used capillaroscopic investigation to evaluate microcirculation damage not as a complication of the disease (diabetes), but as its beginning, and therefore to make a diagnosis (scleroderma).

Our study shows that capillaroscopy is a reliable method for studying periodontal microcirculation. Capillaroscopy makes it possible to study *in vivo* the microcirculatory characteristics of the gingival mucosa and to highlight the significant difference between smokers and non-smokers.^{3,16,3,18}

The smoking habit is an important risk factor in periodontal diseases. In recent years, many studies have tried to evaluate the effects of smoking on microcirculation.

A knowledge of microscopic anatomy is fundamental for the interpretation of the oral vascular examination.¹⁹ In fact, the thickness and typology of the epithelial covering, as well as the presence or absence of keratinization, are directly involved in determining microcirculation visibility and capillary length.^{3,4,16}

In the area of the masticatory mucosa, there was some difficulty in highlighting the vascular bed. This was attributed to surface keratinization and to the remarkable reduction in the extension of the area examined. Due to the architectural characteristics close to the papilla, where the capillary course is mostly parallel to the surface, pathological modifications are more easily detectable. On the contrary, close to marginal gingiva, only the top of the loops can be observed, so morphologic alterations are difficult to detect. For this reason, it was not possible to observe the presence of tortuosity in the area of marginal gingiva. Such a result does not prove the absence of tortuosity due to the perpendicular course of capillary loops in relation to the surface.

In our study, a significant relationship between smoking habit and the presence of capillary tortuosity emerged.

A pathological situation is characterized by an architectural confusion or by the presence of evident morphological anomalies, as shown in fingernail capillaroscopic studies.^{3,7,13,17,20}

Melkonian et al²¹ reported that the smoking habit causes an abnormal pattern formation of chorioallantois membrane blood vessels in chicks, which alters the composition of the extracellular matrix in the chorioallantois membrane mesoderm.

Microhemorrhages represent a very rare phenomenon in both sites of examination. Capillary caliber is significantly reduced in smokers; this statement agrees with the data present in literature.^{18,22-24} According to these studies, nicotine has no direct effect on vascular caliber, but it may interact with certain intravenous substances (norepinephrine, acetylcholine, adenosine phosphate), consequently determining vascular constriction.^{18,23,24} A greater number of capillary loops in marginal gingival was observed, and this must be related to capillary course with respect to the surface.

This study shows that the chronic smoking habit induces significant changes in gingival capillary morphology, caliber and number, which means that such a habit has a determining role as a risk factor in the etiopathogenesis of periodontal diseases.

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