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# Diabetic Microagiopathy of Oral Mucosa Depends on Disease Duration and Therapy

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
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**Background:** Diabetes mellitus is a chronic degenerative systemic disease whose prevalence is increasing. This paper aims to evaluate the effects of diabetic microangiopathy, depending on its duration and the type of treatment administered, by using polarized light videocapillaroscopy of the oral mucosa.

**Material/Methods:** We enrolled 120 subjects: 60 healthy subjects and 60 patients with diabetes mellitus. In turn, patients were divided into 3 subgroups according to the type of diabetes, the duration of the disease, and the type of treatment administered. A videocapillaroscopic examination of the oral mucosa was carried out on the diabetic and healthy subjects.

**Results:** Changes in microcirculation were detected in diabetic patients: at the level of the labial, buccal, and lingual mucosa, the density of the loops is on average reduced; there is an increase in the length and the total diameter of the loops, while the average density of the periodontal capillaries is much higher. The most significant changes were noted in patients who had had type 1 diabetes for more than 10 years and had received insulin therapy.

**Conclusions:** This study, performed using polarized light videocapillaroscopy, which for the first time was used to analyze the capillaries of the oral mucosa in patients with diabetes, confirms the presence of changes that are instrumentally "objectifiable" and "quantifiable" through the videocapillaroscopic technique. Videocapillaroscopy can be a reliable method in the study and monitoring of complications in patients with type 1 and 2 diabetes mellitus.

**MeSH Keywords:** Dentistry • Diabetes Complications • Microcirculation • Microscopic Angioscopy

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## Background

Most diabetic dysfunction occurs at the level of the endothelial cell, with repercussions on vascular permeability, on the metabolism of lipoproteins, on the basal membrane, and on coagulation processes [1–3]. The main function of endothelium is homeostasis through the synthesis and release of a wide variety of coagulants, vasoconstrictors, and vasodilating substances (e.g., clotting factors, prostacyclin, endothelin, prostaglandins, and nitric oxide). These substances modulate vascular tone, vascular permeability, and coagulation. Intact endothelium provides a barrier against the development of atherosclerosis. This protective barrier results in the maintenance of a smooth vascular surface that prevents thrombogenesis, adhesion between monocytes/macrophages, platelets, and the transport of lipoproteins in the cell wall. In a hyperglycemic environment, this barrier is reduced and the reactive mechanisms become dysfunctional, resulting in micro- and macrovascular complications [4–6]. To date, numerous studies in patients with type I and II diabetes have tried to establish changes in circulation in skin through the use of angiometric tests, Doppler, and conjunctival and nail capillaroscopy. The results of these studies have highlighted variations in diabetic patients in the diameter and increased tortuosity of the capillaries in proportion to the duration of metabolic decompensation. Other studies have shown that changes in the microcirculation of the conjunctival mucosa, as observed *in vivo* through capillaroscopy, are always present before retinal angiopathy (once considered an early sign of diabetes) [7–14].

The present study aimed to determine through an *in vivo* assessment of the microcirculation of the oral mucosa whether the effects of diabetic microangiopathy regress, increase, or remain the same depending on the duration of the illness and the type of therapy administered.

## Material and Methods

The experimental study in question took place over a period of 12 months.

We enrolled a total of 120 subjects, divided into 2 different groups: 60 healthy subjects (31 men, 29 women; mean age 55 years, SD 14.52; range: 20–80 years) and 60 patients with diabetes mellitus (31 men, 29 women; mean age 57 years, SD 15.52, range 23–86 years). These patients were in turn divided into 3 subgroups, according to the duration of the disease and the type of treatment administered:

1. 20 patients with type 2 diabetes (8 men, 12 women; mean age 63.05 years, SD 11.44; range: 48–84 years), suffering from the disease for less than 10 years, treated with oral hypoglycemic agents.
2. 20 patients with type 2 diabetes (12 men, 8 women; mean age 67.89 years, SD 10, range 51–86 years), afflicted by the disease for more than 10 years, treated with oral hypoglycemic therapy and insulin.
3. 20 patients with type 1 diabetes (11 men, 9 women; mean age 40.13 years, SD 7.63; range: 23–52 years) who have had the disease for over 10 years, subjected only to insulin therapy.

Instead, the healthy subjects were homogeneously selected according to the mean age and sex of the diabetic subgroups. These were included in the study after a thorough examination of their medical history, by means of which systemic or local diseases which might endanger the local microcirculation were excluded (e.g., connective tissue and diabetes), and by objective examination of the oral mucosa to exclude the presence of lesions or alterations in the oral cavity. None of the patients had dentures (Table 1).

All patients gave their informed consent for the processing and use of personal medical data in scientific papers, in accordance with Italian law.

Each videocapillaroscopic examination took about 15 minutes. After having collected the administrative and clinical data, an intra-oral instrumental examination was performed with the subject in a sitting position, with the same light source (a 6500°K medical neon light), at the same room temperature (23±1°C), in the morning, and by the same operator (GAS). The instrumental examination was repeated at least twice for each site analyzed (Figure 1).

**Table 1.** Criteria for the inclusion of patients enrolled in the study.

| Age range | Non-diabetic control group | Diabetic cases | Duration of the disease | Therapy |
|---|---|---|---|---|
| 48–84 yrs. | 20 HS | 20 PTS with DM2 | <10 years | Metformine |
| 51–86 yrs. | 20 HS | 20 PTS with DM2 | >10 years | Metformine + insuline |
| 23–52 yrs. | 20 HS | 20 PTS with DM1 | >10 years | Insuline |
| Total | 60 HS | 60 patients | | |





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**Figure 1.** A videocapillaroscopic investigation of the labial mucosa.

The areas examined for all the patients were: the lower labial mucosa, the right buccal mucosa, the lingual body, and the periodontal mucosa (gum and interdental papilla).

Two independent observers looked at all the pictures, performing double-blind evaluations for the subgroups. To limit intra-and inter-examination variables, the 2 observers evaluated the same randomly selected images twice.

They considered the following parametric data:
- The density of the capillary loops (the number of loops visible per square millimeter),
- Total length of the capillary loops and their size,
- Incoming and outgoing capillary diameter (the maximum diameter of each of the 2 heads of the circuit),
- The degree of tortuosity of the capillary loops,

and the following non-parametric data:
- Visibility,
- Orientation of the capillaries,
- Presence/absence of microhemorrhages,

- Presence/absence of capillary loops with atypical morphology.

It is important to emphasize that the parametric data originate from software related to videocapillaroscopy using a dedicated measuring instrument, with each optical magnification corresponding to an exact value of metric pixels in the scanned image.

The capillaroscope used was a latest-generation polarized light videocapillaroscope, which in the framework of this experimental study was applied for the first time at the level of the oral mucosa. What distinguishes a polarizing videocapillaroscope from an optical one is the presence of a polarizing filter placed between the light source and the area to be examined. This converts the light from non-polarized to polarized. To obtain polarized light, suitable polarizing filters are used linearly. These are composed of plates that are spaced in the order of the wavelength of the incident light. The plates prevent or dampen the oscillation of the electrical field in the direction perpendicular to them by selecting the polarization parallel to them, forcing the electrical charges to emit wave trains in an orderly manner. The polarization of the light allows certain light radiation to be discriminated, with the aim, for example, of eliminating reflections from reflecting surfaces or lowering the brightness of some objects. The apparatus consists of a handpiece, 3–4 cm in diameter and 11 cm long, weighing 180 g, equipped with microfocus to bring the area being examined into exact focus and allow the operator to work easily in all directions. At the working end, there is a high-resolution camera equipped with a contact scanning system and epiluminescence immersion; microillumination provided by a white light LED; high brightness achromatic optics with micro focus; and a 30× optical zoom with the possibility to get up to 150× magnification through the magnification module, with the horizontal and vertical resolution down to 0.4 µm. The non-working end connects directly to a computer via a single USB cable (3.0), through which it receives power and provides the digital information. The company provides software (Videocap Horus 100 VCS, Adamo srl.) that makes acquiring and reporting data both fast and easy. In particular, the "dynamic positioning" function allows you to manage the follow-up of all lesions in the body wholly or in part. Among the advantages of using the new tool in comparison to the previous one, the most noteworthy are: better visibility; the high resolution of static and dynamic images; the ease in transporting the equipment; and the possibility to connect it directly to a laptop computer, greatly facilitating acquisition maneuvers, storage and retrieval of data.

Two types of static (or morphological) parameters were examined:

1) Non-parametric data:

a) **Visibility of the loops**, depending on the degree of difficulty in bringing the capillaries into focus. The following points were awarded:
   - score 1: Simple focusing, achieved in less than 30 seconds into the examination
   - score 2: Average simplicity in focusing, obtained between 30 seconds and 2 minutes into the examination
   - score 3: Difficult focusing, obtainable in more than 2 minutes from the start of the examination
   - score 4: Focusing impossible.

b) **Orientation with respect to the surface**. The following codes are assigned in relation to the orientation of the capillaries to the surface:
   - code A: Capillaries parallel to the surface
   - code B: Capillaries perpendicular to the surface
   - code AB: Capillaries are both parallel and perpendicular to the surface.





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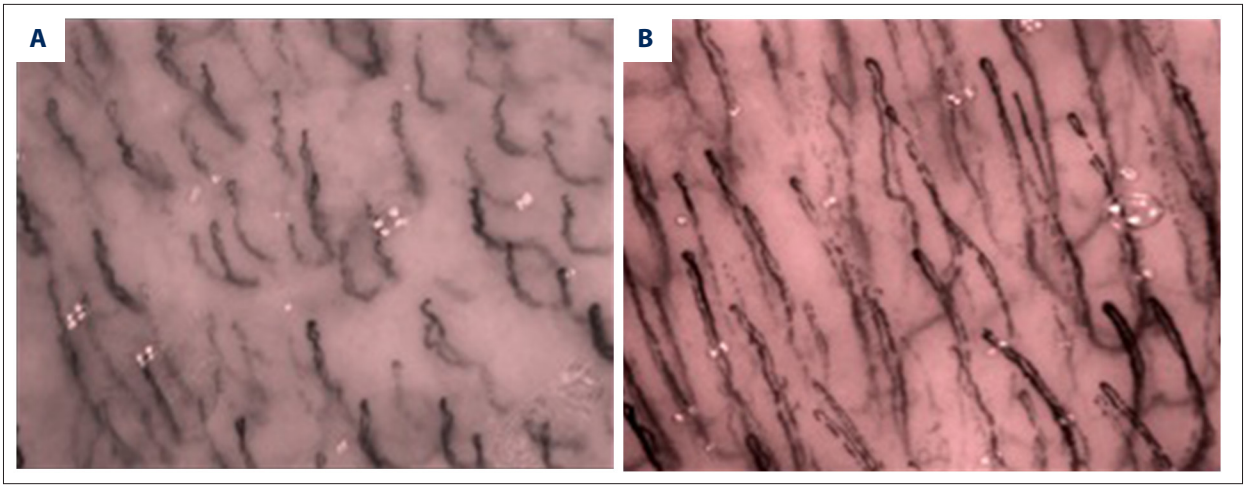


**Figure 2.** Example of buccal mucosa in type 2 diabetics treated with metformin for less than 10yrs/healthy subjects.

c) **The presence of microhemorrhages**, attributing the following scores:
   – 0: Absence
   – 1: Presence
d) **The presence of capillaries with characteristic morphology, attributing the following scores**:
   – 0: Absence
   – 1: Presence

2) Parametric data:
Parametric data come from software connected to the videocapillaroscope through a specifically calibrated system. The following parameters are therefore accurately obtained:

a) **Length of the capillary loop is** one of the most difficult parameters to determine because it is conditioned by the projection of the vessel on the mucosal surface. The more parallel the flow is to the surface, the longer the capillary will seem and vice versa. In any case, the normal range of the length of a capillary varies between a minimum of 150 µm and a maximum of 500 µm (for rectilinear capillaries).
b) **Diameter of the loop.** This is generally between 4 and 14 µm, with differences between the arteriolar branch, which is thinner, and the venular one, which is thicker. The first should have a diameter of about 8 µm, the second 10 µm, and the intermediate portion, the loop itself, 8–14 µm.
c) **Capillary tortuosity**, obtained by assigning a score from 0 to 3, according to the number of crossings present (0=no crossing, 1=single crossing, 2=more than 2 crossings, 3=distorted loops).
d) **Capillary density**, number of loops present per $mm^2$ (on average between 12 and 20).

## Results

In total, more than 2400 capillaroscopic images were examined (4 sites investigated for each of the 120 subjects and a minimum of 5 frames for each area examined), and for each image a minimum of 5 measurements were taken, for a total of over 12 000 measurements.

The degree of significance was set at $P<0.05$.

Subgroup 1 labial mucosa: capillary density 15.04±3.35 (diabetics), 20.86±2.53 (healthy) $P<0.05$; length 0.253±0.063 (diabetics), 0.198±0.069 (healthy) $P<0.05$; total diameter 0.039±0.007 (diabetics), 0.032±0.008 (diabetics) $P<0.05$.

Subgroup 1 periodontal mucosa: capillary density 34.61±11.56 (diabetics), 16.55±4.38 (healthy) $P<0.05$;
Subgroup 2 labial mucosa: capillary density 14.62±3.35 (diabetics), 21.17±6.54 (healthy) $P<0.05$;
Subgroup 2 periodontal mucosa: capillary density 34.92±11.67 (diabetics), 16.78±4.98 (healthy) $P<0.05$;
Subgroup 3 periodontal mucosa: capillary density 35.25±10.23 (diabetics), 18.20±5.73 (healthy) $P<0.05$ (Figuress 2–5).

## Discussion

Diabetes mellitus is a chronic disease and has important effects on peripheral macro- and microcirculation [15–18].

An alteration in the endothelium has negative consequences on vascular permeability, on the metabolism of lipoproteins, at the expense of the basal membrane, and on the clotting process.

Periodontal vascularization is greatly affected by the progression of diabetes mellitus. It is enough to consider that





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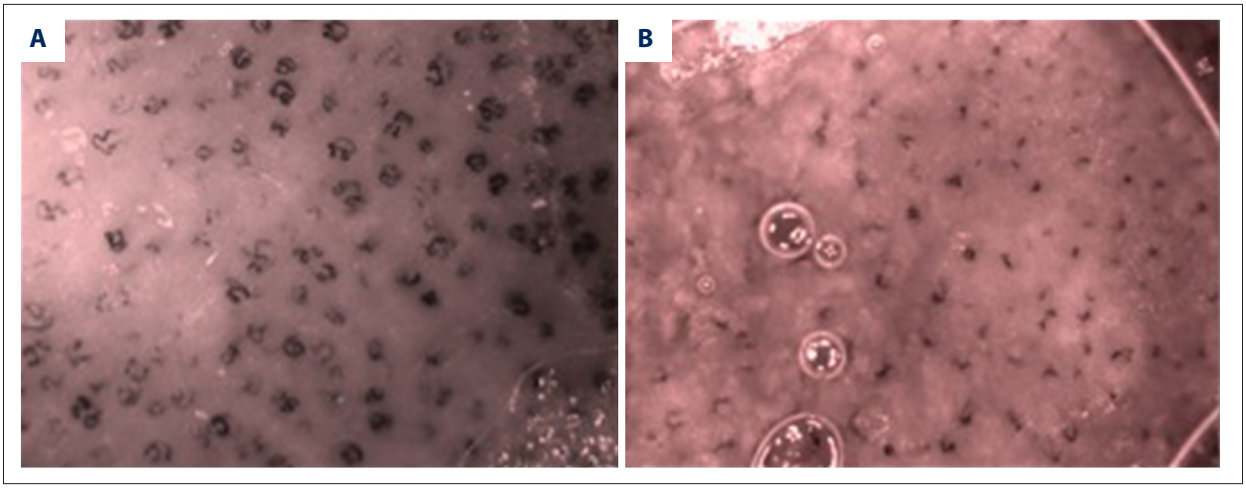


**Figure 3.** Example of microcirculation in the gingival mucosa of type 2 diabetics treated with metformin for less than 10 years/healthy subjects.

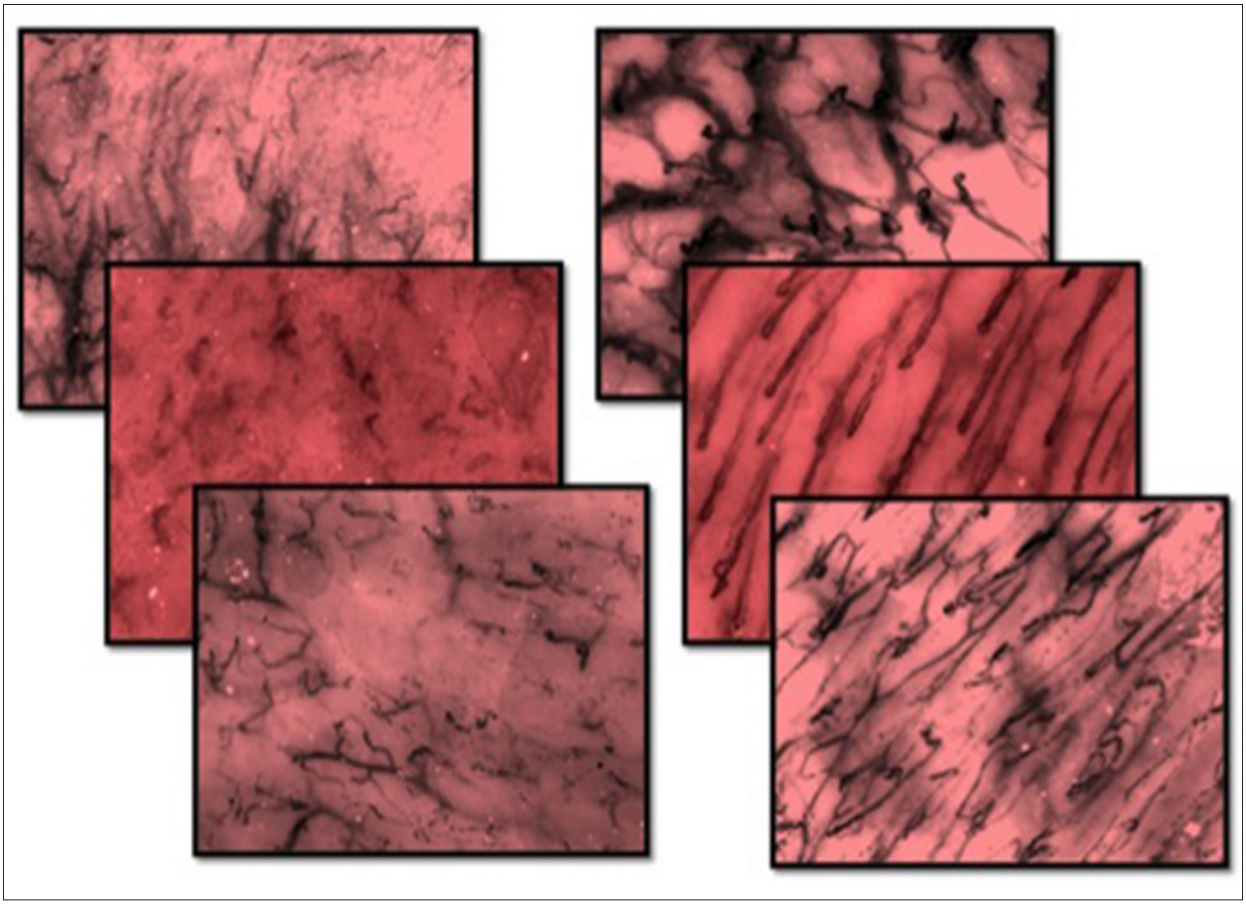

**Figure 4.** Example of labial microcirculation, and microcirculation in the buccal mucosa and the lingual body of type 1 diabetics treated with insulin for more than 10yrs/ healthy control group.

periodontal disease represents the sixth most important complication and that a diabetic is twice as likely to develop periodontal disease independent of the effect of age and local factors (16).

The dysfunction in the endothelial vessel is also reflected at the capillaroscopic level, as seen from the results of numerous studies in the literature [7–10,13–15].





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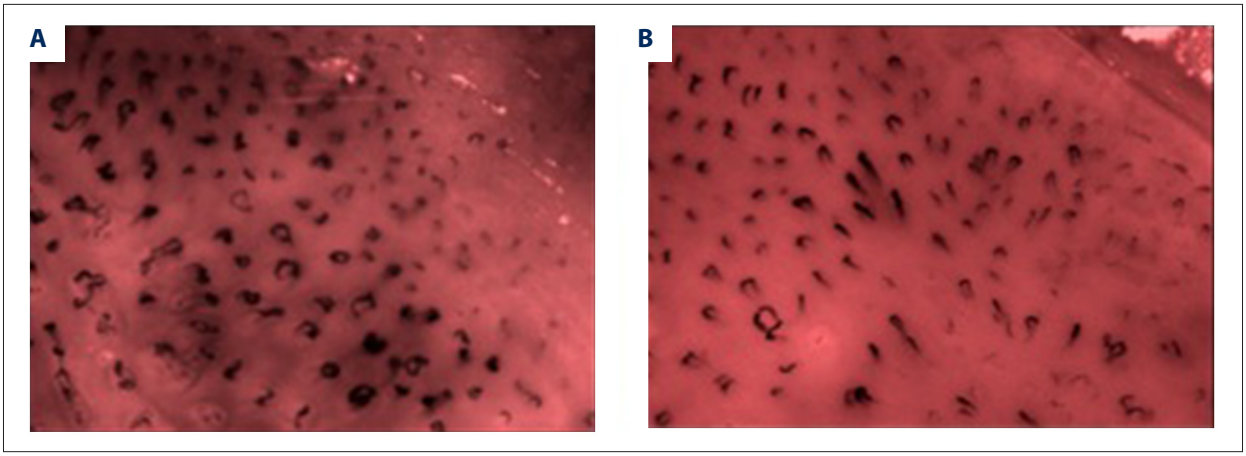


**Figure 5.** Example of periodontal mucosa in type 1 diabetics treated with insulin for more than 10 years/healthy subjects.

This study actually confirms the great impact that diabetes has on the peripheral vascular system.

Also in this case, the generalized reduction in capillary density that is found at the level of the labial, buccal, and lingual mucosa of diabetics, compared to the control group, can be considered a symptom of peripheral microangiopathy, which is typical in these patients.

As in other anatomical areas, diabetic microangiopathy also induces a reduction of the peripheral tropism in the oral mucosa.

This reduction in microcirculation at the oral level, together with the concentration of salivary glucose, could explain, at least in part, the increased susceptibility to infections caused by opportunistic pathogens such as *Candida albicans*.

Apart from the decrease in capillary density, a widespread increase in the average length and total diameter of the loops was also observed [7,13].

In essence, the capillary loops in diabetic patients on average seem longer and wider than in healthy patients.

The distance between the 2 extremities, both between the incoming and outgoing points and in relation to the apex of the loop, tends to widen until assuming a flat appearance, or a morphology called “staghorn”.

All this can be interpreted as an attempt by the body to compensate for the reduced tropism of the mucosa by increasing the metabolic exchange surface between the circulation and the tissue, in response to the reduction in capillary density.

The result of this attempt is the production of compensatory longer capillaries, which are more tortuous and have a wider head, the latter constituting the part most involved in metabolic exchanges, thanks to a thin fenestrated epithelial lining [15,18].

It should also be pointed out that the diameter of the 2 ends of the circuit undergoes no statistically significant change, in contrast to what happens in chronic inflammatory pathologies such as oral lichen planus, rheumatoid arthritis, and Sjögren’s syndrome.

Therefore, there are no signs of increased tissue perfusion, but there is a greater exchange surface.

With regard to the vascular bed of the superficial periodontium (masticatory/gingival mucosa), there was found to be a net increase in capillary density in diabetics compared to healthy subjects.

It was confirmed that the density of blood vessels in diabetic subjects is about twice that of healthy subjects, which is synonymous with an intense angiogenic activity.

Another feature of the periodontal capillary model in diabetic subjects is the typical “leopard spot” morphology, with widespread microhemorrhages and capillaries arranged in a rosette formation.

This study, therefore, demonstrates that, if applied to the analysis of the superficial periodontium, videocapillaroscopy is important for the early diagnosis and monitoring of periodontal disease, offering the possibility of objectifying *in vivo* a specific periodontal microcirculatory pattern in subjects with diabetes, regardless of the presence of clinical signs and symptoms that are more or less visible, such as spontaneous or induced bleeding and an increase in the flow or an alteration of the composition of the sulcular fluid [7,13].





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The innovation introduced by this experimental study to the knowledge in the medical-dental field consists in having described for the first time the relationship between treatment patterns and the objectifiable effects of diabetic microangiopathy at the level of the oral mucosa.

Given that there is a correlation between the density of capillaries and oral diabetic microangiopathy in all patients with diabetes, the data show that there are significantly better conditions in patients receiving effective therapy for glycemic control compared with decompensated subjects.

In contrast, a decline in microcirculatory health was noted in patients who had been suffering from type 2 diabetes mellitus for more than 10 years and who were forced to use insulin due to the inability to achieve adequate glycemic control by using only oral hypoglycemic agents.

This information has implications for clinical dental practice, especially in the surgical field (implantology), which an increasing number of elderly people with type 2 diabetes mellitus resort to [12,17].

The recent, remote, and pharmacological medical history of the patient is therefore essential in order to completely understand the risk of a patient incurring probable complications during the healing of the surgical site [12].

All this shows how important it is to have early diagnosis, to devise an exactly appropriate treatment regimen, and to adopt appropriate preventive measures in the approach to these patients.

The most evident signs of diabetic microangiopathy were observed in patients with type 1 diabetes mellitus, particularly in cases where there had been an onset of the disease at an early age.

These are patients who have lived with the disease for a long time (more than 10 years), subjected for obvious reasons (being an insulin-dependent diabetic) to insulin therapy alone. It is in these subjects that there is the highest incidence of microvascular complications.

Patients with type 1 diabetes mellitus are those that have the highest susceptibility to alterations in microcirculation, a delay in bone regeneration, and microangiopathy in gingival areas.

Moreover, the evidence shows that the prevalence of microvascular complications in patients with type 1 diabetes mellitus is higher in patients older than 20 years compared to those who are under 10 years old [18].

## Conclusions

Ultimately, our research indicates that videocapillaroscopy can be a reliable method in the study and monitoring of complications in patients with type 1 and 2 diabetes mellitus, since the data show significant changes in the morphology, size, and density of capillaries in microcirculation in the oral cavity. This is probably correlated to the therapeutic regimen and the duration of the disease.

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