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The Cutaneous Microvascular Architecture of Human Diabetic Toe Studied by Corrosion Casting and Scanning Electron Microscopy Analysis

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ABSTRACT

In this morphological study, we report on the three-dimensional microvascular architecture constituting the toes of a patient affected by diabetic microangiopathy. We applied corrosion casting (CC) technique to the toes of a patient affected by Type 2 diabetes, who underwent surgery for explantation of inferior left limb due to necrotic processes of soft tissues. The toes of a foot traumatically explanted in a motorcycle accident were kept as controls. According to technical protocols, toes were injected with a low-viscosity acrylic resin (Mercox) through the major digital artery, tissues were corroded in KOH solution (8%), and resulting casts processed for SEM observations. Already at low magnification, in diabetic toes, we found an impairment of the linear track-like disposition of the vessels of plantar side, with signs of vascular disruption and obliterations, stopped resin, and leakages. Capillaries under the nail and a lot of vascular villi in eponychium and nail borders were damaged, and vascular regression phenomena acting on them were clearly visible. Resin leakages and impairment of normal vascular architecture were also observed in the root of the nail. This preliminary report represents only the first step for further investigations regarding morphological three-dimensional appearance of diabetic microangiopathy. CC and scanning electron microscopy technique well documented these morphological modifications, highlighting on both structural and ultrastructural features of diabetic toes microvessels. In conclusion, our qualitative data try to better focus on the pathophysiological mechanisms involved in diabetic dermopathy and microangiopathy, proposing CC as useful method to investigate on them. Anat Rec, 293:1639–1645, 2010. © 2010 Wiley-Liss, Inc.

Key words: human digit; Type 2 diabetes; corrosion casting technique; scanning electron microscope

Noninsulin-dependent diabetes mellitus (NIDDM), also called Type II diabetes, is one of the most common diseases affecting elderly people; it is often combined, under the term "metabolic syndrome," with hypercholesterolemia and hypertension. The systemic consequences caused by excessive glucose plasma levels and the production of free radicals and advanced glucose end products are peripheral neuropathy, microangiopathy, and

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dermopathy. Diabetic dermopathy has been termed the most common cutaneous finding in diabetes, occurring in as many as 40% of diabetic patients older than 50 years.

The impairment of normal microcirculation, mostly visible at the capillary level, may lead to a gradual loss of cutaneous trophism, discromia, and later necrosis and ulcers. These modifications have always been thought to be mainly caused by microvascular alterations and in particular by the occlusion of superficial capillaries due to microthrombotic events and consequent vascular regression phenomena.

These pathological conditions have always been studied using laser Doppler technology as to demonstrate that diabetic dermopathy lesions are the result of a cutaneous ischemic process (Rendell et al., 1989; Rendell and Bamisedun, 1992; Aso et al., 1997). Although patients with diabetic dermopathy exhibit reduced skin blood flow and volumes, it is still a matter of debate whether diabetic dermopathy represents a local ischemic process or not (Netten et al., 1996; Urbanic-Rovan et al., 2004; Wigington et al., 2004).

Moreover, a different distribution of blood between skin capillaries and subpapillary vessels in the toes of diabetic patients has been demonstrated in capillary blood cell velocity (CBV) and laser Doppler fluximetry (LDF). The ratio between CBV and LDF was found to be lower in diabetic patients (Jorneskog et al., 1995a,b; Jorneskog and Fagrell, 1996).

In our study, a morphological three-dimensional microscopic technique was used to document the impairment of microcirculation in diabetic dermopathy in one of the most peripheral vascularized cutaneous sites, fundamental for its thermoregulatory and tactile functions: the human toe (Conrad, 1971; Bryce and Chizuka, 1988; Forst et al., 2006).

As demonstrated in previously published work (Sangiorgi et al., 2004), the corrosion casting technique combined to scanning electron microscopy (SEM-CC) clearly documents the microvascular architecture of the cutaneous area in the normal digit, being able to describe the three-dimensional disposition of capillaries, their shape, and orientation (Grant and Bland, 1930; De Takats, 1932; Hale, 1951; Edwards, 1960; Miyamoto, 1963; Straile, 1969; Baden, 1970; Hundeiker, 1971; Blanka and Alter, 1976; Backhouse, 1981; Achten and Parent, 1983; Misumi and Akiyoshi, 1984; Moss and Schwartz, 1985; Pollit and Molyneux, 1990; Nasu et al., 1998).

This method consists of a precasting treatment (to clean the vascular lumen and remove all the blood), injection of the casting medium, corrosive treatment of the specimens, dissection, mounting, coating, and finally observation by SEM.

The aim of this work is to give a preliminary qualitative report on cutaneous microvascular modifications we found in the toes of a single patient affected by diabetes Type II, NIDDM.

MATERIALS AND METHODS

All the procedures were performed in accordance with ethical rules for the use of personal data.

Surgical Procedures

The lower limb of a 75-year-old patient (a) affected by Type 2 diabetes was surgically explanted because of necrotic processes, which had occurred to soft tissues. The foot of a 33-year-old patient (b), explanted after a motor accident and no longer reimplantable, served as a control.

Shortly after surgery, all toes were explanted, the vascular peduncles were exposed under a dissection stereomicroscope, and a 24 G cannule inserted in the major lateral artery of each one. The cannule was fixed in the vessel by silk ligature and connected to a three-way infusion system.

Corrosion Casting Procedure

The precasting treatment consisted of an intravascular injection of 10 mL of heparinized saline solution to prevent blood clotting. The pressure of the injection was kept constant and monitored using a manometer connected to the infusion system ($p = 20$ mmHg). Attention was paid not to cause any interstitial edema (swelling of toes during injection). A second injection of 5 mL of saline solution was performed to remove all the blood cells and to wash the heparinized solution out of the vascular bed, thus preventing any damage to the endothelial cell lining that could modify the cells morphology or soften their intercellular junctions.

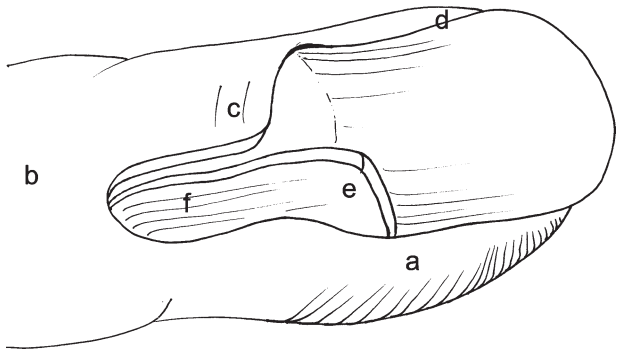
The first step consisted of a low-viscosity resin injection (5 mL of Mercox) previously mixed with benzoyl peroxide as a catalyzer. The infusion rate was kept constant using an automatic infusion peristaltic pump (2 mL/min) until the reflux from the venous vessels became evident and the pressure of injection increased (the pressure was kept constant equal to 20 mmHg). The afferent and efferent vessels were then closed with metallic staples and the toes immersed in hot water (60°C) to complete the hardening process. Afterward all the toes underwent a corrosive process in KOH solution (8%) at room temperature; the solution was changed every 12 hr for 4–5 days. The obtained casts, once cleared of tissues, were washed in distilled water and dissected under a stereomicroscope to obtain small specimens of different cutaneous areas and then prepared for SEM observation (Murakami, 1971; Hodde and Nowell, 1980; Miodonski et al., 1980; Weiger et al., 1986; Castenholz, 1989; Lametschwandtner et al., 1990; Rolf and Nilsson, 1992).

SEM Observation

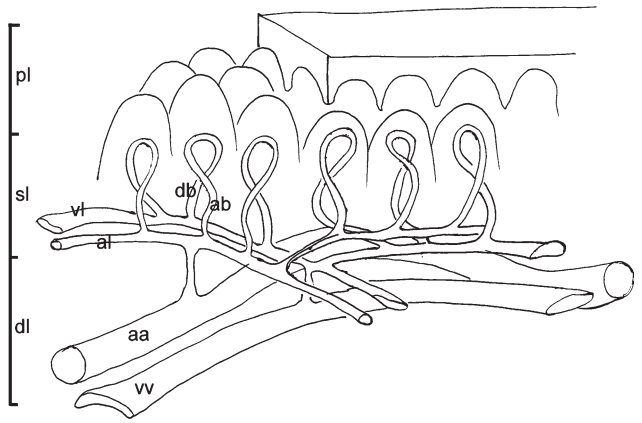
The resulting casts were rinsed in distilled water, dehydrated with a fast bath (2 min) in a solution of 100% alcohol, critical point dried in an Emitech K850 CPD apparatus, coated with 10 nm gold in an Emitech K250 sputter coater, and observed by a Philips XL-30 FEG SEM working at 10 keV. Some specimens, because of their dimensions, needed metallic conductive bridges to prevent specimen charging and to improve image quality.

RESULTS

We analyzed each toe both of the patient a (diabetic) and b (control): six different cutaneous areas were identified (all the studied areas are represented in the



Scheme 1. Graphical representation of a human toe evidencing all the investigated areas: plantar side (a), dorsal side (b), eponichium (c), perionichium (d), nail bed (e), and nail root (f). Note that the nail and the skin of eponichium have been partially removed to allow a better visualization of nail bed and nail root.



Scheme 2. Graphical representation of vascular layer constituting the human toe skin: dermal layer (dl), with big arteries (aa) and veins (vv); subpapillary layer (sl) characterized by arterioles (al) and venules (vl); papillary layer (pl) characterized by vascular villi made up of an ascending branch (ab) and a descending branch (db).

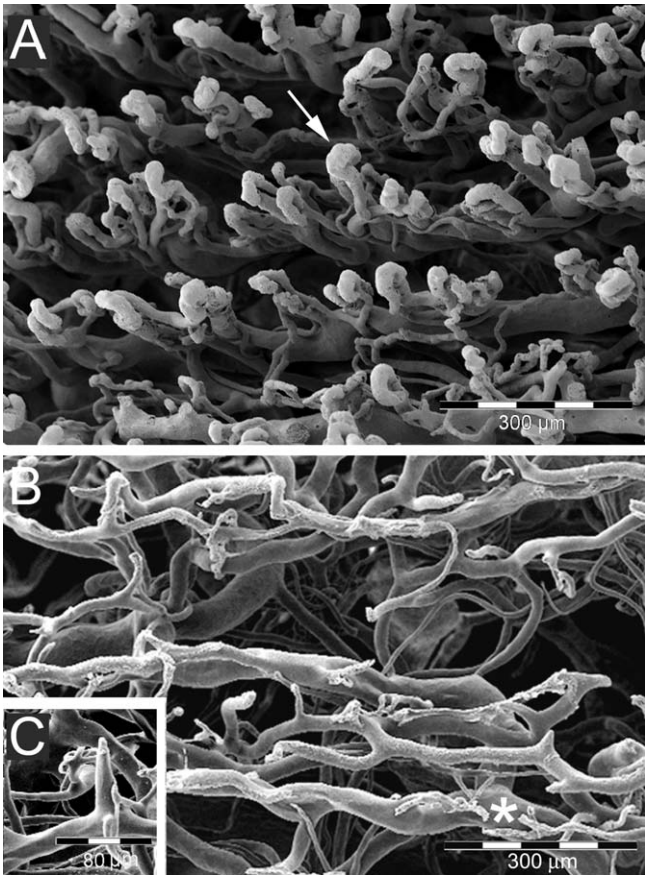


Fig. 1. Plantar side: (**A**) Normal toe: at low magnification it is possible to observe the two track-like disposition of subpapillary vessels giving rise to many vascular villi with dextrogirate orientation (arrow). (**B**) Diabetic toe: note the absence of vascular villi (only the first ascending part is visible) and the normal microvascular structure of underlying vascular layers made up of medium-sized veins and arteries (*). (**C**) At high magnification, it is possible to better visualize the interrupted cast at the level of ascending branch of a vascular villi.

graphical Scheme 1): plantar (a) and dorsal side (b) of toe skin, eponychium (c), perionychium (d), nail bed (e), and nail root (f).

To be better oriented when looking at cutaneous microvasculature, we represented a graphical scheme (Scheme 2) evidencing:

- the dermal layer (d) with large-sized arteries (aa) and veins (vv) running freely in the dermal layer;
- the subpapillary layer (sl) characterized by arterioles (al) and venules (vl);
- the papillary layer (pl) characterized by vascular villi (vv) made up of an ascending branch (ab) arousing from the arteriole and a descending branch (db) draining into a venule.

Plantar Side—Area (a)

The microvascular architecture of the plantar side in the normal toe is made up of medium-sized feeding arteries and draining veins running freely in the dermal layer from which two track-like vessels originate that strictly follow the orientation of fingerprints (subpapillary layer) (Fig. 1). From these vessels, dextrogirate vascular villi (consisting of an afferent ascending capillary and an efferent descending one) arise and enter each dermal papillae (papillary layer) (Fig. 1A). In the diabetic toes of patient (a), at low magnification it was already possible to observe an impairment of the shape and disposition of these vessels: the track-like dermal subpapillary vessels become more tortuous even if they seem to maintain the same orientation as the control (patient b) following the direction of fingerprints.

The vascular villi arising from the longitudinal vascular ridges are lost or highly damaged (Fig. 1B). Sometimes the ascending branch is visible ending with a "mouse tailed" or roundish tip, clear evidence of resin stop, probably due to vascular microthrombotic processes (Fig. 1C).

Dorsal Side—Area (b)

The microvascular architecture of the dorsal side in the normal toes is characterized by the absence of a

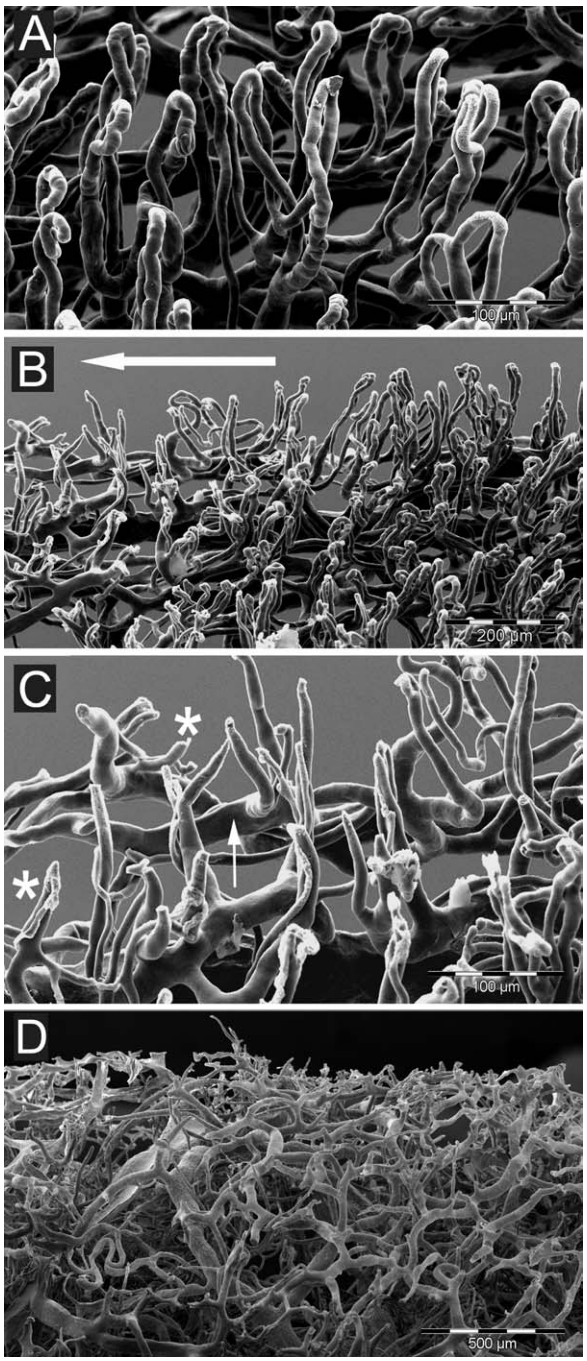
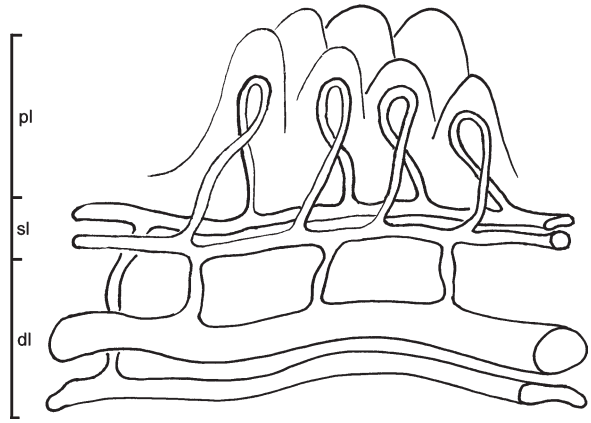


Fig. 2. Dorsal side: (**A**) In the normal toe, it is possible to observe dermal vascular villi formed of an ascending capillary and a descending capillary organized in a dextrogirate structure. (**B**) The vascular villi that usually enter the dermal papillae gradually disappear going caudal-cranially along the major axis of the digit (arrow). (**C**) At high magnification, it is possible to document the vascular obliteration of ascending capillaries of vascular villi (*) that seem to be interrupted. When reaching the eponychium, we can no longer observe any vascular villi. However, the underlying dermal microvascular structure characterized by medium-sized vessels is clearly visible (arrow). (**D**) The eponichium in diabetic toe is characterized by the complete absence of vascular dermal villi. The underling vascular architecture is well visible.



Scheme 3. Transverse section of toe normal skin: note the distribution of papillary (pl), subpapillary (sl), and dermal (dl) vessels.

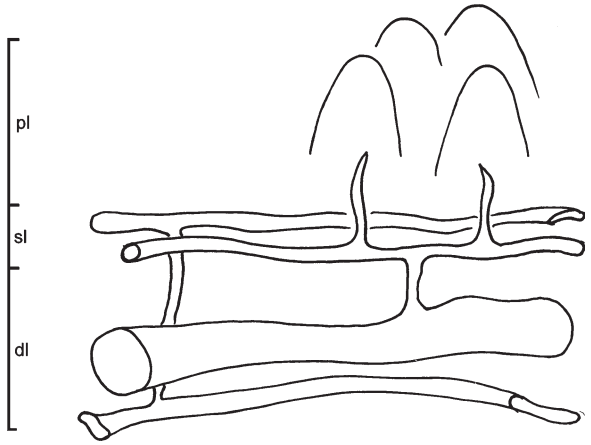
constant direction of dermal subpapillary vessels because of the absence of fingerprints (Fig. 2A–C). The dextrogirate vascular villi arising from them are higher than those found in the plantar side, but they maintain the same direction, perpendicular to subpapillary layer, entering dermal papillae (Fig. 2A). In diabetic toes, these villi appear to be damaged and sometimes interrupted as in palmar side (Fig. 2B). Moreover, it was also possible to observe the loss of their efferent descending branch while the ascending branch is characterized, as already observed on the plantar side, by "mouse tailed" or roundish tips (Fig. 2C). To better distinguish the normal pattern of dermal microvasculature from the diabetic one, we made two graphical schemes simplifying what previously observed by SEM analysis of casts: in the first, we represented the normal distribution of dermal, subpapillary vessels and dermal villi seen in a transversal section (Scheme 3); in the second, the ascending capillary of vascular dermal villi is interrupted, whereas the subpapillary and dermal microvasculature is preserved (Scheme 4).

Eponychium—Area (c)

The eponychium is the cutaneous site closest to the origin of the visible part of the nail (Fig. 2D). In this area, the thickness of the dermal layer gradually decreases caudal-cranially, while the dermal papillae lengthen and the angle they form with the major axis of the digit decreases. As a consequence, the microvascular structure of the eponychium in the normal toe is characterized by an increase in the height of vascular villi until reaching the visible part of the nail where they disappear and continue on the surface in contact with the nail as a wide net of randomly arranged capillaries. In the diabetic toes, the vascular villi of the eponychium are lost, and the underlying dermal vascular net becomes more visible (Fig. 2D).

Perionychium—Area (d)

The vascular structure of the perionychium in normal toes is made up of a wide net of interlaced capillaries that form a thin vascular sheet next to the borders of the nail bed without any vascular trabeculae or villi. No



Scheme 4. Transverse section of toe diabetic skin: note the interruption of the ascending branch of vascular villi in papillary layer (pl) and the normal distribution of vessels in subpapillary (sl) and dermal (dl) layers.

particular features were noted in the diabetic toes compared with the normal ones.

Nail Bed—Area (e)

In the nail bed of normal toes, it is possible to see parallel vascular trabeculae directed along the major axis of the digit in the direction of the growing nail that follow dermal ridges (Fig. 3A). In the diabetic toes, the vascular trabeculae become more tortuous and convoluted (Fig. 3B). At high magnification, it is also possible to see clear signs of intercellular extravasations in the form of conglomerates or plastic sheets lying among the cast vessels (Fig. 3C). Sometimes it is also possible to see the incomplete filling of the resin in the trabeculae, which demonstrates the obliteration of the vessels (Fig. 3D).

Nail Root—Area (f)

The microvascular architecture of the nail root in normal toes is made up of a dense net of longitudinal capillaries running toward the nail bed and gradually turning into their vascular trabeculae (Fig. 4A). At high magnification, it is also possible to observe some dome-shaped sprouts on the cast capillaries. In the diabetic toes, these vessels appear to be more tortuous and it is no longer possible to distinguish the dome-shaped sprouts (Fig. 4B,C).

Analyzing the subpapillary layer in all these areas, we could also observe some arteriovenous shunts either in the form of laterolateral or end-to-side arteriovenous anastomosis. It was also sometimes possible to observe extravasated conglomerates of resin in the form of sheets lying on the cast or spheroid bodies among them (Fig. 5).

DISCUSSION

In this study, we reported on the observation we made on the three-dimensional microvascular modifications occurring to papillary and subpapillary capillaries in the toes of a single patient affected by diabetic dermopathy and microangiopathy.

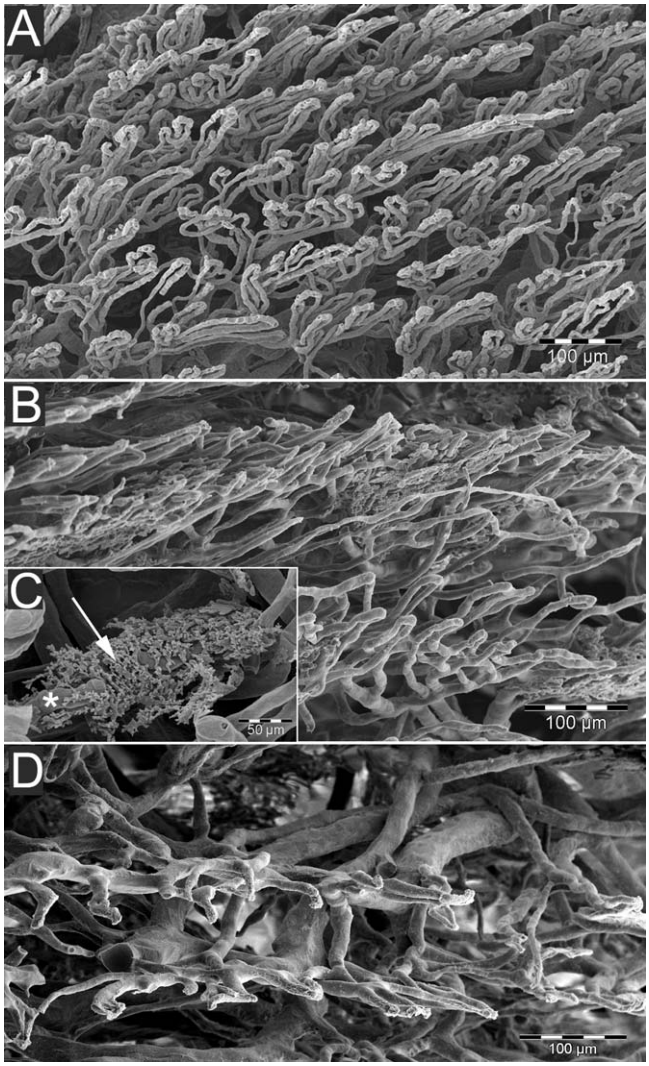


Fig. 3. Nail bed: (**A**) The normal disposition of vascular trabeculae made up of vascular villi entering the dermal papillae all oriented along the major axis of the digit. (**B**) Low magnifications of vascular trabeculae in diabetic toe: (**C**) note the presence of vascular leakages around capillaries and, at high magnification (arrow), (**D**) some interrupted vessels of vascular villi.

We decided to use CC technique and SEM analysis because, even if it is not completely free of artifacts, it is the most widely used technique to analyze the microvessels both in normal and pathological conditions at high definition and in three dimensions.

The impairment of the capillary architecture in six different cutaneous regions of human diabetic toes was documented. Qualitative modifications, occurring mostly to microvascular villi, were observed in all the areas.

Microvascular villi in the diabetic toes maintain the same topographic disposition in three dimensions depending on the area observed but appeared to be mostly interrupted, probably because of vascular regression phenomena or microthrombotic processes that commonly affect capillaries of diabetic patients. This fact is well documented by CC-SEM analysis: the resin is

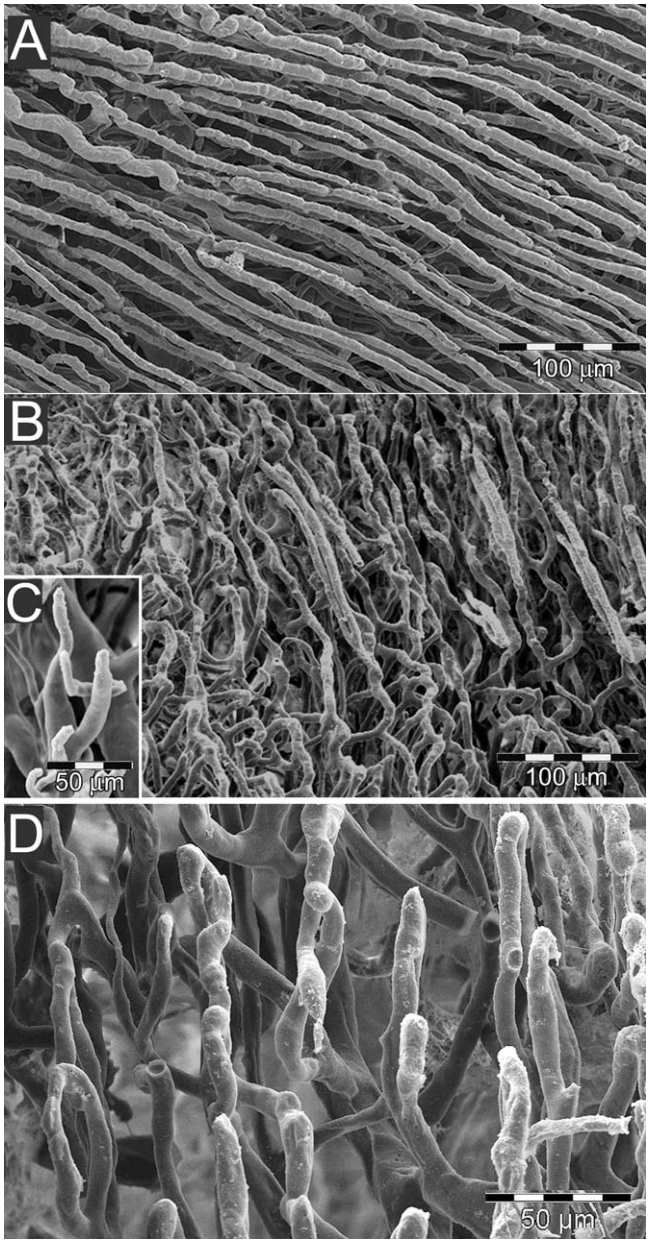


Fig. 4. Nail root (**A**): in the normal toe, the capillaries of the nail root are straight and directed along the major axis of the digit (arrow) and ending in the trabeculae of the nail bed previously described. (**B**) In the diabetic toe, the disposition seems to be more disorganized and present convolution of vessels. (**C**) Also, some sign of vascular obliteration are evident. (**D**) At high magnification, the dome-shaped sprouts visible in normal toe are no more distinguishable.

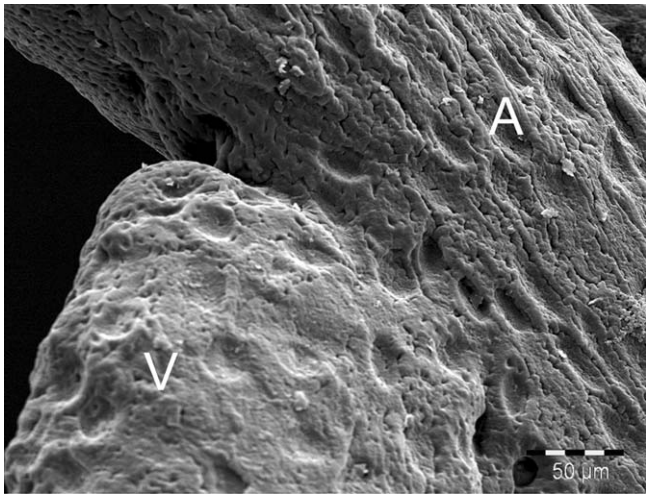


Fig. 5. Arteriovenous connection: note the disposition of the imprints of endothelial cell nuclei: on veins (V) they are arranged randomly along the vessels, whereas on arteries (A) they are placed along the major axis of the vessel. This difference enables us to distinguish arteries from veins and to demonstrate this end-to-side arteriovenous anastomosis.

stopped and does not fill in the entire terminal vessel forming the so-called "mouse tail" shaped vessels.

The disruption of cellular membranes of endothelial cells, the breakdown of their junction and the consequent impairment of physiological lining is witnessed by the presence of intercellular leakages, visible as resin sheet lying on the vessels.

Moreover, we could observe conglomerates of resin surrounding the casts or flake-like structures probably caused by the diffusion of the resin into the lymphatic vessels or in interstitial tissues.

In the dermal layer, it was also possible to observe some arteriovenous anastomoses that can be interpreted as signs of open collateral vascular circuits. This data, if confirmed by further investigations, could support the results obtained in the study of perfusion in diabetic foot microvessels *in vivo*: in the feet of patients with diabetic neuropathy, total skin blood flow has demonstrated to be increased because of a shunt flow mechanism.

In this study, we focused our attention mostly on the superficial papillary and subpapillary vascular networks and in particular on the capillaries entering the dermal papillae.

The distribution of these capillary alterations is patchy, and this condition reflects the distribution of cutaneous discromiae, one of the most common signs of diabetic dermopathy.

On the plantar side, the area most involved in tactile function impairment, we can observe vascular regression phenomena of vascular papillary villi that could result in ischemic processes affecting also nerve endings and dermal bodies.

Also, in the nail root, we could observe a modification both in the orientation and in the integrity of capillary vessels, which are probably signs of a reduction of capillary oxygenation and the consequent trophism of the nail, often observed in diabetic patients as nail morphological modifications. Moreover, the angiogenic sprouts found in the nail root of the normal toe are absent.

All these results document a distal, capillary impairment already demonstrated by previous authors as a reduction in tissue oxygenation at the cutaneous level and in our study demonstrated by a morphological qualitative three-dimensional point of view.

The CC method was able to document in three dimensions the modifications occurring in the microvessels of human diabetic toes to better understand the

mechanisms underlying diabetic dermopathy and microangiopathy and its consequences.

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