

Three-dimensional Architecture of Human Cutaneous Vascular Lesions: A Scanning Electron Microscopic Study of Corrosion Casts

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Corrosion casts were made from angiosarcoma of the scalp of an elderly person, Klippel-Trenaunay syndrome, pseudo-Kaposi's sarcoma, diffuse neonatal hemangiomas, cavernous hemangioma and arteriovenous malformation and were examined by scanning electron microscopy (SEM). The normal scalp from a cadaver was studied as a control for the angiosarcoma of the scalp.

The SEM of the vascular corrosion casts clearly demonstrated the fine three-dimensional (3-D) architectures of malignant and benign cutaneous vascular lesions; the superficial fine and medium-sized vascular networks of the skin were replaced by abnormal balloon-like dilatations, extravasated fringes, glomerular structures, sinusoidal vessels or localized bulla-like structures of the capillary loops, depending on the nature of the cutaneous vascular lesions. The corrosion cast technique is helpful for understanding the 3-D patterns of various vascular lesions. **Key words:** *angiosarcoma; hemangioma; vascular malformation; angioarchitecture.*

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Classifications of cutaneous vascular lesions have been based on clinical descriptions, anatomical configurations, histological findings and hemodynamic characteristics (1–3). In addition, three-dimensional (3-D) architectural differences among them have been studied by conventional angiography (4, 5). However, the finer 3-D architectures of these lesions still remain uncertain even in these studies.

Cutaneous angiosarcoma is an aggressive malignancy which usually occurs on the scalp and face of elderly people (6, 7). From a therapeutic standpoint, it is considered to be important to know the 3-D patterns and modes of the growth of the neoplasm.

The purpose of this study was to demonstrate the 3-D architectural patterns and the relationship with the pre-existing vessels in human cutaneous malignant and benign vascular lesions. Vascular corrosion casts were examined by scanning electron microscopy (SEM) (8, 9), and the results were compared with the clinical and histopathological features.

MATERIALS AND METHODS

Vascular corrosion casts were obtained from 6 human cutaneous vascular lesions, including angiosarcoma, Klippel-Trenaunay syndrome, pseudo-Kaposi's sarcoma, diffuse neonatal hemangiomas, cavernous hemangioma and arteriovenous malformation. The normal-looking temporal scalp from a 61-year-old male cadaver was used as a control for the angiosarcoma of the scalp.

Immediately after surgical excision of the specimens, feeding vessels were exposed under the operating loupe, a small caliber cannula was inserted and properly fixed with a thread. The specimens were perfused

with 0.9% saline through the cannula to wash out the intraluminal content, followed by injection of a casting medium (Mercor CL-2R; Dainippon Ink & Chemicals Inc., Tokyo, Japan) mixed with 2% catalyzing substance. After finishing the injection, the cannula was left in place and the specimen was kept for 12 h at room temperature to allow the injected medium to solidify (polymerize). Then the injected tissue was macerated for several days in a solution of 20% KOH, interrupted by a short, daily rinse in tap water for removal of tissue remnants. The corrosion casts thus obtained were washed in running water for one day and dried in air at room temperature. The blocks of the corrosion casts were mounted on the specimen holders with silver paste (Silver P-255; Nisshin EM Inc., Tokyo, Japan), coated with platinum in an ion coater (IB-5; Eiko Engineering Inc., Tokyo, Japan) and observed under a SEM (S-800; Hitachi Inc., Ibaragi, Japan) at an accelerating voltage of 5 kV.

Small portions of the lesions were also prepared for light microscopy. The tissue pieces were fixed in 10% formalin, dehydrated through a series of graded concentrations of ethanol and embedded in paraffin. Paraffin sections, approximately 6 μ m thickness, were stained with hematoxylin-eosin and were examined. The diagnosis of each lesion was based on the clinical and histopathological findings.

RESULTS

Vascular corrosion casts satisfactorily demonstrated fine, 3-D vascular beds in each cutaneous vascular lesions except for partial filling defects. The normal-looking temporal scalp of the cadaver showed rich superficial, medium-sized vascular networks and finely branching smaller vessels (Fig. 1), though the

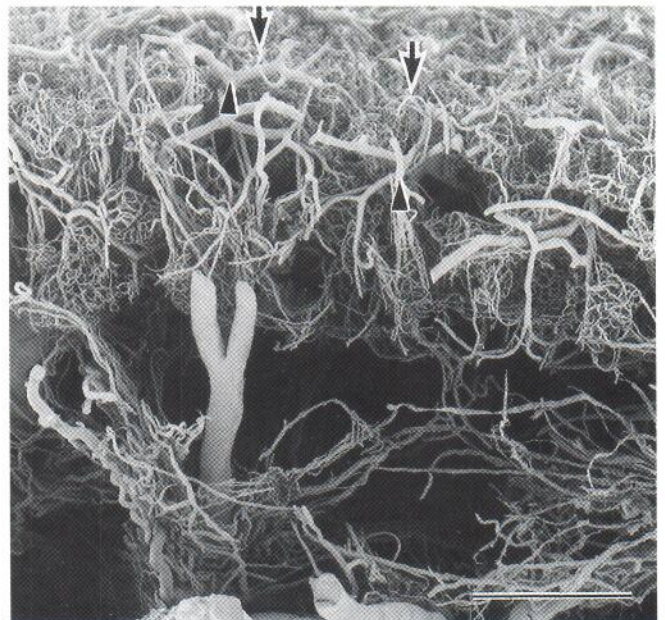


Fig. 1. Scanning electron micrograph of a vascular corrosion cast of the normal temporal scalp of a 61-year-old male cadaver, 6 h after death, viewed from the side. Superficial vascular networks consist of interconnecting medium-sized (arrow heads) and finely branching smaller vessels (arrows). No capillary loops are seen. Bar, 1 mm.

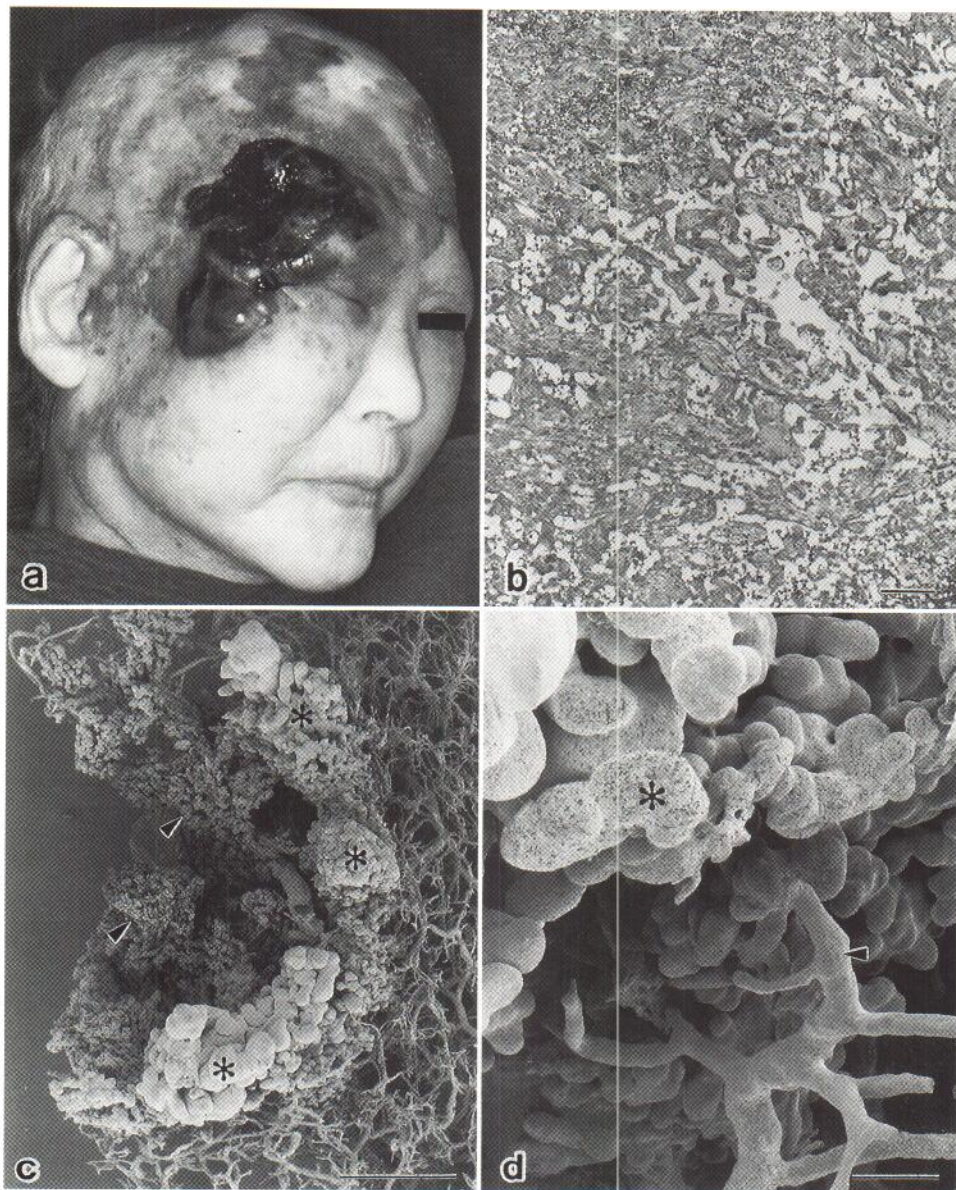


Fig. 2. Angiosarcoma. (a) 80-year-old female, 1 month before death. An erythematous macule had developed on the frontoparietal region of the scalp 2 years earlier. The lesion recurred following a wide local excision and extensive radio-chemotherapy. (b) Light micrograph showing irregular vascular clefts, hemorrhages, and trabeculae lined by large, atypical endothelial cells (hematoxylin-eosin; bar, 0.1 mm). (c) Scanning electron micrograph of a vascular corrosion cast, viewed from the top. Clusters of balloon-like nodules (*asterisks*) replace the superficial vascular networks. *Arrow heads* indicate clusters of minute fringes, which correspond to the extravasated cast material. *Bar*, 1 mm. (d) A higher magnification of (c), viewed from the below. Multilocular clustered balloon-like vessels (*asterisk*) connected with the underlying larger vessels (*arrow head*). *Bar*, 0.1 mm.

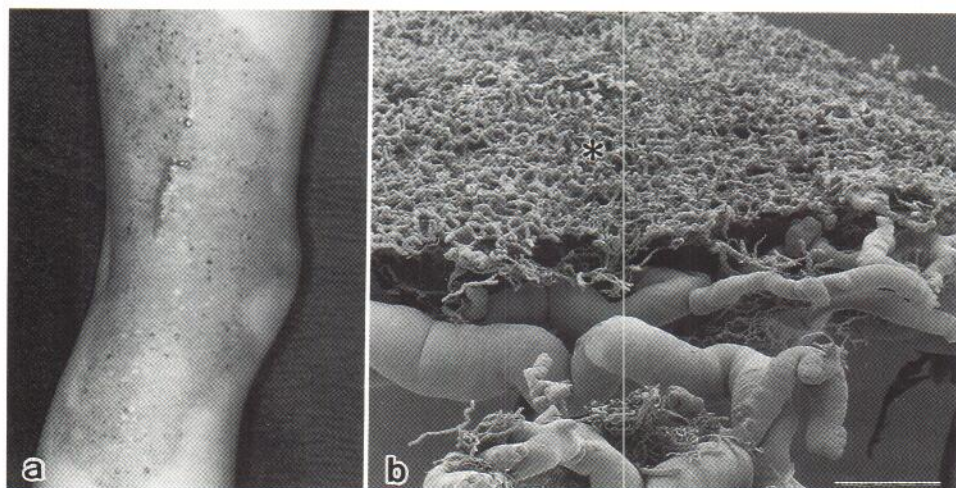


Fig. 3. Klippel-Trenaunay syndrome. (a) 7-year-old female. Well-demarcated, purplish-red macules on the right lower extremity were noted at birth. Deep-red papules developed later. She also had varicose veins and hypertrophic bone and soft tissues of the affected limb. (b) Scanning electron micrograph, viewed from the side, showing rather dense superficial medium-sized vascular networks (*asterisk*) and underlying markedly dilated, tortuous vessels. *Bar*, 1 mm.

Fig. 4. Pseudo-Kaposi's sarcoma. (a) 26-year-old male, showing brown to deep purplish macules and papules on the dorsum of the right foot. Angiograms revealed multiple arteriovenous fistules of the affected limb. (b) Light micrograph showing a marked proliferation of capillaries in the papillary layer and dilated vascular lumina in the middle dermis (hematoxylin-eosin; bar, 0.1 mm). (c) Scanning electron micrograph of a vascular corrosion cast, microdissected so that the superficial fine networks are partially removed, viewed from the top. Bar, 1 mm. (d) A higher magnification of (c) before dissection, showing globular swelling (arrow heads) of the tips of the superficial capillary loops. Bar, 0.1 mm.

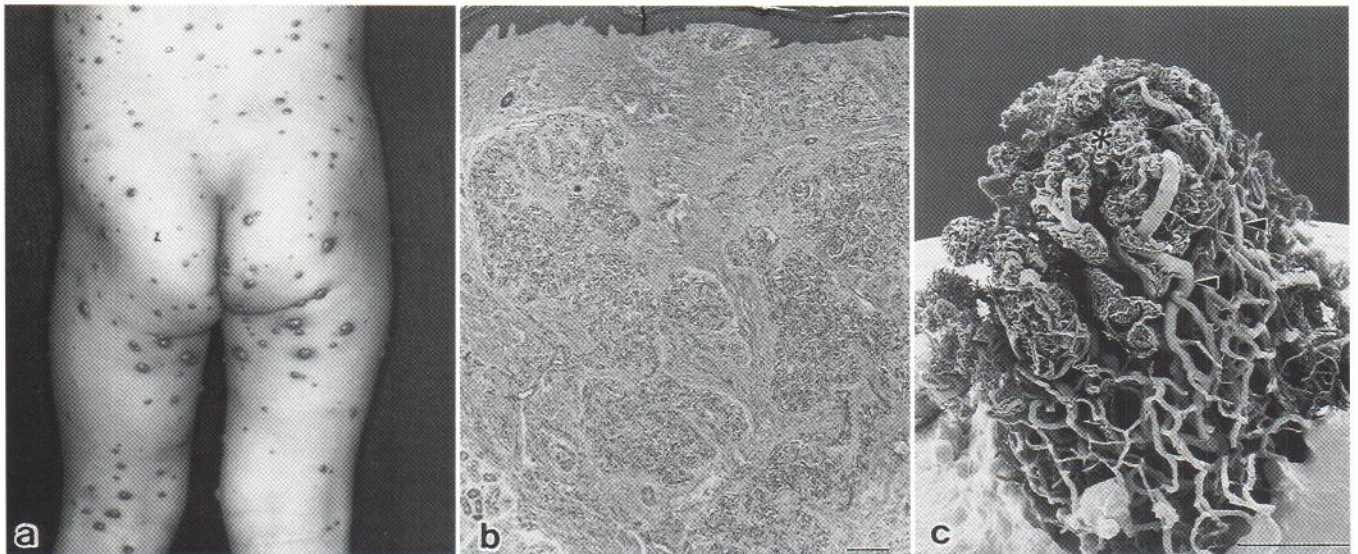
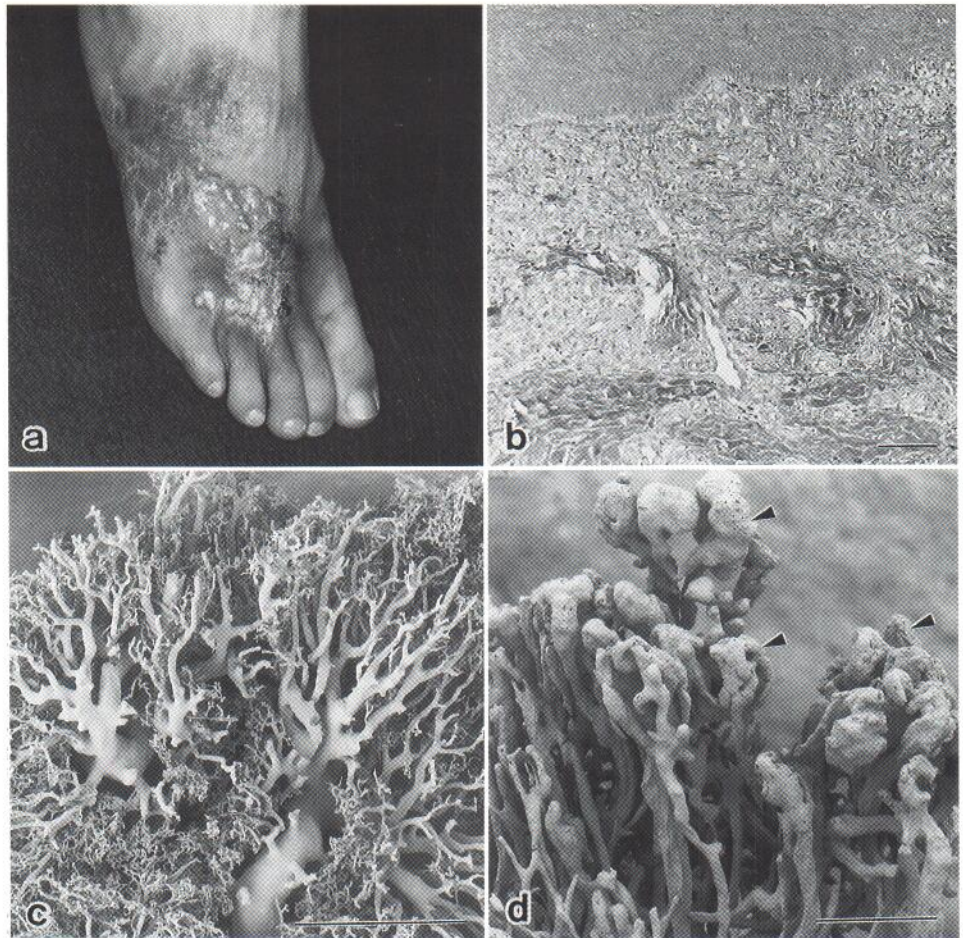


Fig. 5. Diffuse neonatal hemangiomas. (a) 3-year-old female. Numerous cherry-red papules on the trunk and extremities, and a huge cavernous lesion in the axilla were found at birth (10). The number and size of the papules kept increasing with intracranial vascular lesions. (b) Light micrograph of a small lesion, showing multiple circumscribed lobules of capillary proliferation (hematoxylin-eosin; bar 0.2 mm). (c) Scanning electron micrograph, viewed from the side, showing protruding, multilobular, glomerular, smaller vessels (asterisk), interconnected with medium-sized vessels beneath (arrow heads). Bar, 1 mm.

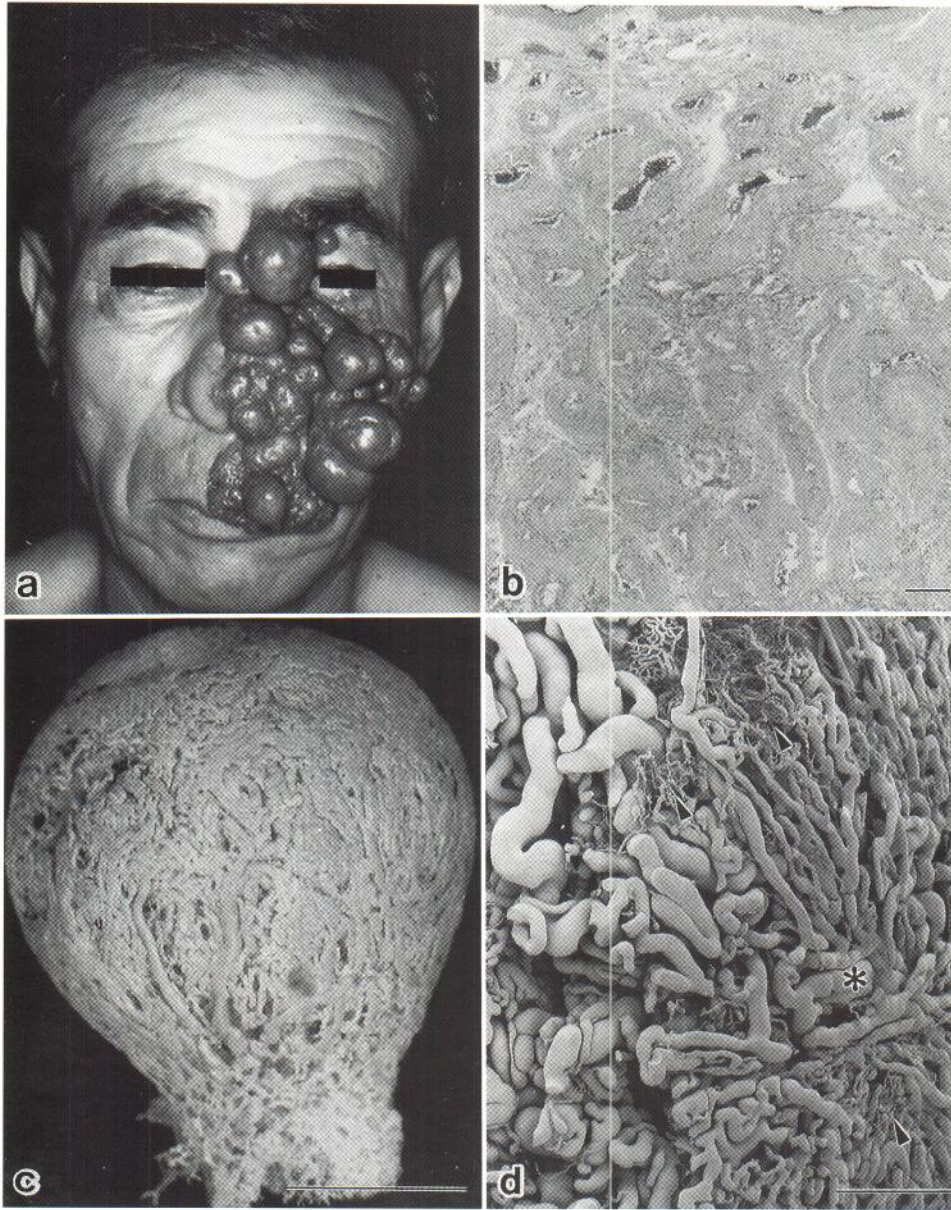


Fig. 6. Caverosus hemangioma. (a) 58-year-old male, showing multiple nodules developing in a large port-wine hemangioma of the face. (b) Light micrograph, showing multiple, closely packed, thick-walled vessels (hematoxylin-eosin; bar, 0.2 mm). (c) Macroscopic appearance of the whole vascular corrosion cast. Bar, 10 mm. (d) Scanning electron micrograph, viewed from the top. Tortuous and sinusoid vessels (*asterisk*) are closely packed together with a few finer vessels (*arrow heads*), which show localized bulla-like structures. Bar, 1 mm.

filling defects were also observed in the subepidermal capillary loops.

Angiosarcoma

A specimen was obtained from the right temporal edge of the bleeding tumor 2 h postmortem with permission (Fig. 2a). Histological examination revealed irregularly communicating vascular clefts, hemorrhages, and trabeculae lined by apparently atypical, large endothelial cells (Fig. 2b). SEM also demonstrated clusters of balloon-like nodules and minute fringes replacing the medium-sized and finely branching, smaller vascular networks (Fig. 2c). The balloon-like nodules corresponded to the irregular vascular clefts and the minute fringes to the extravasated cast material. High magnification revealed that the balloon-like nodules were connected with the underlying larger vessels. No apparent imprints of endothelial cell nuclei were seen on the surfaces of the ballooned areas (Fig. 2d).

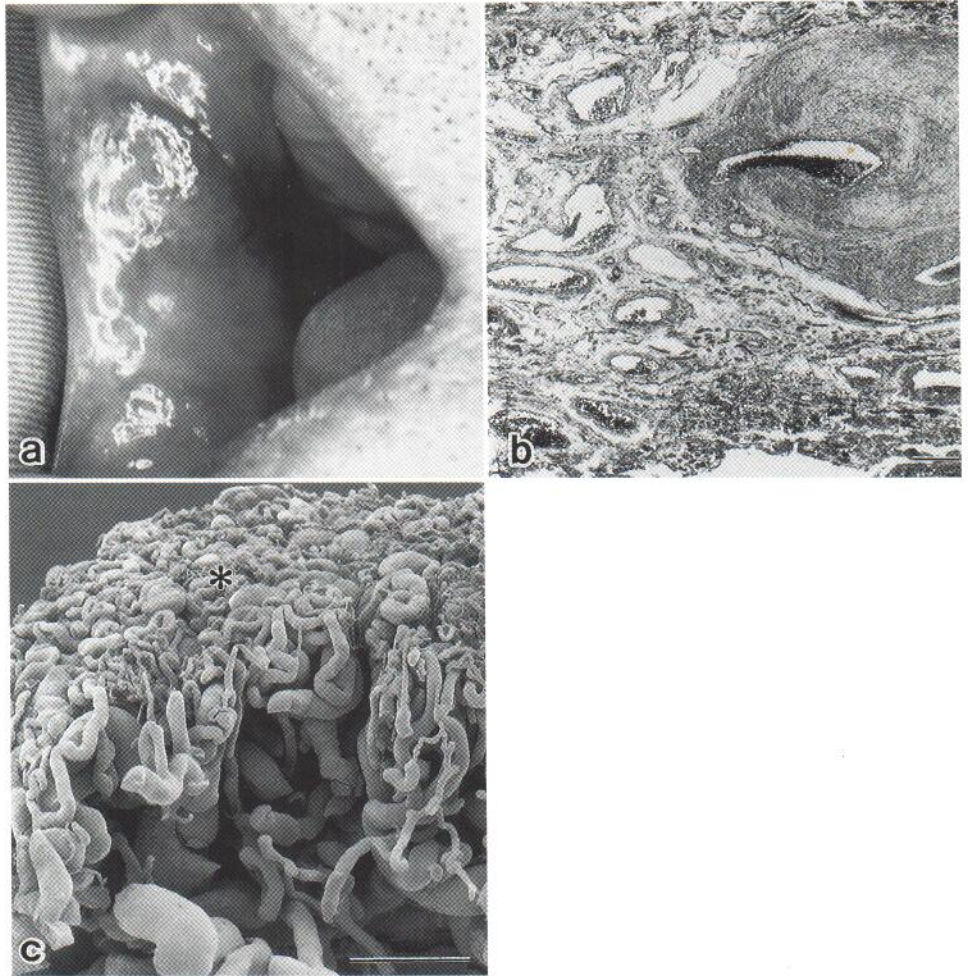
Klippel-Trenaunay syndrome

A specimen was taken from the macule on the lateral aspect of the knee (Fig. 3a). Histological examination showed dilated small vessels in the upper dermis. SEM demonstrated rather dense superficial medium-sized vascular networks, parallel to the skin surface, and underlying markedly dilated and tortuous vessels (Fig. 3b). Capillary loops were scarcely seen.

Pseudo-Kaposi's sarcoma

A specimen was obtained from the brownish macule (Fig. 4a). Histological examination revealed a marked proliferation of capillaries in the papillary layer and dilated vascular lumina in the middle dermis (Fig. 4b). SEM demonstrated superficial networks of finer vessels with larger arborating vascular supplies simulating a coral-like structure (Fig. 4c). The tips of the capillary loops showed globular swelling (Fig. 4d).

Fig. 7. Arteriovenous malformation. (a) Right buccal mucosa of 62-year-old male, showing a dark pinkish pulsating soft nodule, 2.5 × 1.5 cm in size. (b) Light micrograph, showing thick-walled and thin-walled, dilated vessels in the submucosa (hematoxylin-eosin; bar, 0.1 mm). (c) Scanning electron micrograph of a vascular corrosion cast, viewed from the side. Numerous tortuous, dilated vessels of various caliber (*asterisk*) are closely packed and interconnected with underlying larger, dilated vessels. Bar, 1 mm.



Diffuse neonatal hemangiomas (10)

A specimen was taken from the buttock (Fig. 5a). Histological examination revealed multiple circumscribed nodules of capillary proliferation in the dermis, lined by normal-appearing endothelial cells (Fig. 5b). SEM demonstrated protruding multilobular, glomerular structures. The main feeding artery entered the center of the structure from beneath and branched to supply the individual lobule. Draining veins from each lobule appeared to run along the outer surface of the structure (Fig. 5c).

Cavernous hemangiomas

Multiple dark purplish pedunculating nodules were examined (Fig. 6a). Histological examination revealed closely packed, thick-walled vascular lumina (Fig. 6b). Macroscopy of the whole vascular corrosion cast demonstrated a tightly packed wad of various-sized vessels (Fig. 6c), which consisted of numerous tortuous and sinusoid vessels together with finer vessels showing localized bulla-like structures (Fig. 6d). The cut surfaces of the cast were similar to the surface view.

Arteriovenous malformation

A pulsating dark pinkish nodule was removed and examined (Fig. 7a). Histological examination revealed many thick-walled and thin-walled dilated vessels in the submucosa (Fig. 7b). SEM

demonstrated numerous, interconnecting, tortuous, dilated vessels of various caliber with underlying larger, dilated vessels (Fig. 7c).

DISCUSSION

In the past, fine structures of cutaneous vascular lesions have been studied, mostly by histopathological sections. Recently, 3-D analysis has been made by conventional angiography (4, 5) or by the reconstruction model from the serial pathological sections (11). However, the angiograms are not readily available and not detailed enough, and the reconstructions from the serial sections required too elaborate work.

The corrosion cast technique has proven itself to be an excellent procedure for demonstrating the fine 3-D structures of the vascular system, though the possibilities of artifacts from the filling defects, extravasation of cast materials, or from coagulation of the vascular contents should always be kept in mind (8, 9).

Since 1971, vascular patterns in normal and pathological tissues and organs, mostly in experimental animals, have been studied using SEM of vascular corrosion casts (12–20). However, those of human tissues and organs are still insufficiently known (21–25), and human cutaneous vascular lesions have never been subjected to such a study.

The present SEM studies comprised 6 different vascular lesions and the normal scalp of a cadaver. The irregular vascular clefts in angiosarcoma of the scalp showed clusters of balloon-like nodules in the corrosion cast. The imprints of atypical endothelial cell nuclei, however, were not visualized on the ballooned surfaces. This was possibly caused by a failure to fill the vessels in full tension due to the leakage of the cast material, which was demonstrated as minute fringe-like structures. A 3-D corrosion cast study of hamster liver angiosarcoma from carcinogenic substance demonstrated features similar to those of the human scalp angiosarcoma in the present study, such as interconnecting nodules of varying sizes corresponding to the irregular vascular clefts (25).

Port-wine stain in Klippel-Trenaunay syndrome showed rather dense, superficial medium-sized vascular networks in the subepidermal layer, with a few remnant capillary loops. These findings were compatible with those reported in a 3-D reconstruction of a macular telangiectasis made from serial photomicrographs (11). The capillary loops in normal human skin (27) appeared to be replaced by the subepidermal, horizontal vascular networks in the port-wine stain.

The coral-like structure of the pseudo-Kaposi's sarcoma was a quite unique finding. A marked capillary proliferation and its characteristic configurations were considered to reflect a hypoxic condition, occurring distally to the arteriovenous shunt (28, 29).

The histopathological pattern of diffuse neonatal hemangiomatosis was compatible with those of capillary hemangioma. The 3-D architecture, showing lobular or glomerular structures, was also compatible with the angiographic findings in strawberry hemangioma (4). This indicated that the capillary proliferation of the lesions had occurred in lobular patterns.

On angiography, the presence of a main feeding artery was demonstrated in both nodular cavernous hemangiomas and arteriovenous malformation, where the former was of low-flow type and the latter a high-flow one (2). Numerous sinusoid vessels in nodular cavernous hemangiomas in the present study were more closely packed than those seen in arteriovenous malformation. The vascular tortuosity of the latter was less prominent. The architectural differences also appeared to be effected by the hemodynamic differences between them.

In spite of repeated perfusions to remove the intraluminal contents before the injection of the casting medium, the vascular corrosion cast of the normal cadaver scalp showed an incomplete filling of the capillary loops 6 h after death. Although the maximum permissible time after death for obtaining proper vascular corrosion casts has never been clarified, an interval of less than 2 h between death and injection at room temperature has been recommended (30).

Although the types and numbers of the vascular lesions in the present study were quite limited, the 3-D architectures in the vascular corrosion casts appeared to be fine in detail enough to reveal the growth patterns and mode of extension of the lesions, which is helpful in understanding the pathogenesis. Although at this time, studies of this type may have limited practical application, it may be of interest to know as much as possible about these 3-D architectures of cutaneous vascular lesions.

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