Tissues, Pathology, and Diagnostic Microscopy

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Scanning electron microscopy of vascular corrosion casts is still an excellent technique to study microvascular anatomy and vascular pattern formation of tissues and organs in health, disease and experimental conditions.

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In 1971 Murakami [1] for the first time used the scanning electron microscope to study vascular corrosion casts (VCCs) gained by filling the vascular bed of tissues and organs with a polymerizing resin. Because the hardened resin resists tissue maceration by strong alkali as well as removal of bones by hydrochloric acid remaining plastic casts are termed “vascular corrosion casts”.

Since Murakami`s pioneering study the technique was refined in several aspects. These refinements are: (1) Micromanipulator guided fine-tipped glass electrodes allow to cannulate and inject the cardiovascular system of embryos, fetuses, larvae and of animal and human tissue and organ fragments gained during routine surgery or postmortem [2-5]; (2) Resins used – for details see [6-8] – nowadays consist of two components only – a prepolymerized base resin and a catalyst – which easily can be mixed to gain the ready-to-inject-resin; (3) VCCs can be frozen in distilled water and be sectioned in any desired level by a mini-wheel-saw placed in the chamber of a cryo-microtome [9] or can be cut simply by razer blades if casts are made from PU4ii [10]; (4) cut VCCs can be re-frozen in distilled water and subequently freeze-dried to preserve the spatial integrity of even the most delicate vascular territories; (5) VCCs can be quantitatively studied by 3D-morphometry using stereopaired SEM images [11-12] enabling new insights into the construction (optimality) principles underlying the design of cardiovascular systems [13]; and lastly (6) VCCs can be used for microcomputertomography (μ-CT) to study cardiovascular systems without the need to further section or dissect the specimen as virtual images can be reconstructed from data gained from micro- computer tomograms.

At present SEM of VCCs in combination with 3D-morphometry enables to qualitatively and quantitatively study the 3D arrangement of normal and diseased cardiovascular systems from early fetal/larval development to senescence (Figures 1 and 2). In detail, the technique allows (i) to study endothelial surface morphology of vessels by their imprints preserved on cast surfaces (Figure 3), (ii) to analyze branching patters of arterial and capillary vessels in terms of diameters of and branching angles between parent and daughter vessels, (iii) to define interbranching and intervascular distances, (iv) to localize flow regulating structures like flow dividers (Figure 4), intimal cushions, (Figure 5), microvenous valves [14] (Figure 6), and sites of vascular sphincters (Figure 7), and (v) allows to visualize signs of ongoing sprouting (Figure 8) and non-sprouting angiogenesis and its facets invoved in maturation, adaptation and reshaping of vascular territories [2] (Figure 8).

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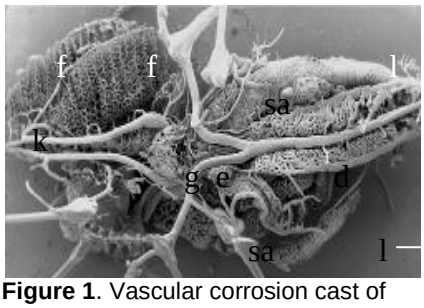

Figure 1. Vascular corrosion cast of a tadpole of the African Clawed Toad, *Xenopus laevis*. Dorsal view. SEM micrograph. Dorsal view. d dorsal aorta, e esophagus, f filterplate, g glottis, k kidney, l lung, sa systemic arch.

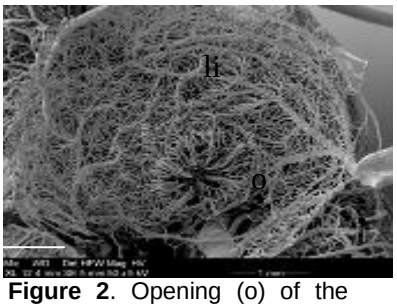

Figure 2. Opening (o) of the small intestine into the large intestine (li) as seen from the large intestine. Adult *Xenopus laevis*.

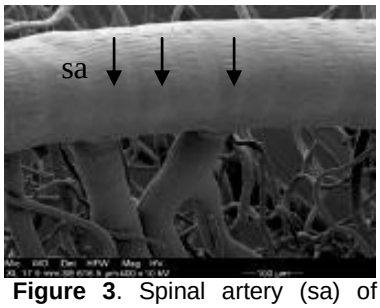

Figure 3. Spinal artery (sa) of adult *Xenopus laevis*. Note the slender longish endothelial cell nuclei imprints orientated parallel to the vessel long axis characteristic for arterial vessels. Arrows indicate circular imprints from vascular smooth muscle cells.

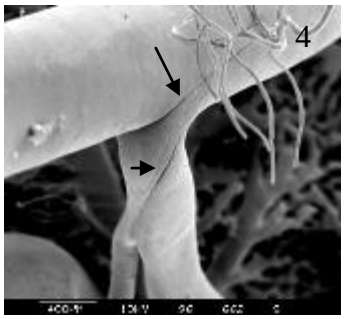

Figure 4. Flow dividers (arrows).

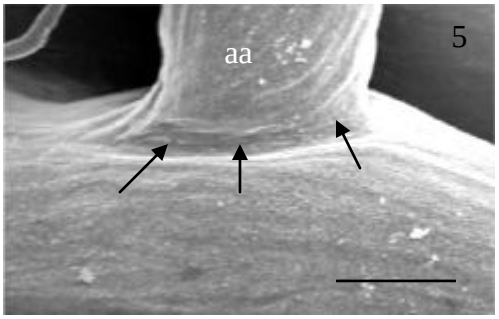

Figure 5. Intimal cushion (arrows) at the origin of an arteriole (aa).

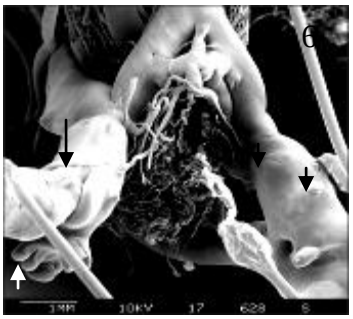

Figure 6. Venous valves of the renal portal vein cast orthogradely (large arrow) and retrogradely (small arrows). Adult *Xenopus laevis*.

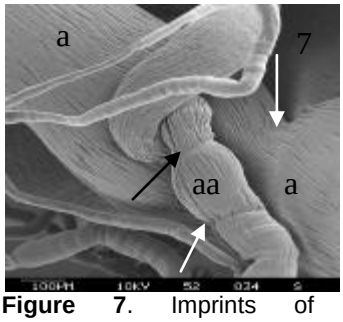

Figure 7. Imprints of muscular sphincters (arrows) on a small artery (a) and an arteriole (aa). Note the characteristic endothelial cell nuclei imprints on artery and arteriole.

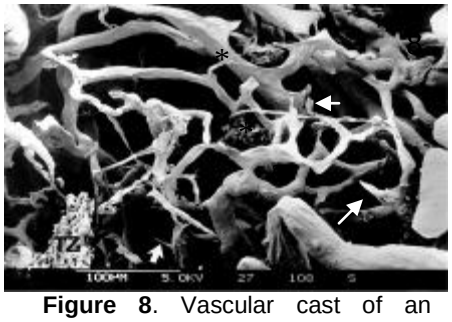

Figure 8. Vascular cast of an experimental glioma in the rat cerebral cortex. Note compressed flattened tumor vessels (asterisks) and vascular sprouts (arrows). a artery, TZ tumor center.

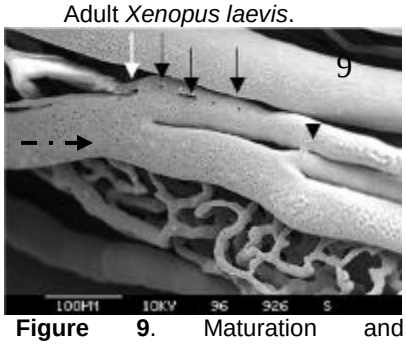

Figure 9. Maturation and remodelling of vascular branching patterns by intussusceptive microvascular growth (IMG). Note first signs of formation and ingrowth of tissue pillars (arrows) which will lead to a new side branch whose origin will be shifted distally. A small anastomosis (arrowhead) is still present between two closely neighbouring distal branches of the same vessel. If this anastomosis is preserved and enlarges or if it regresses cannot be defined yet. Dashed arrow indicates proposed direction of blood flow.