Recent advances in scanning electron microscopy of vascular corrosion casting and their importance in microvascular research

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Vascular casting is an old technique used to demonstrate the three-dimensional architecture of the blood vascular system (for history see [1-4]). Initially, specimens were fragile, non-durable, and primarily visualized the macrovasculature. The use of polymerizing resins [5-9] which withstand the corrosive action of alkali and acids led to durable and easyto-handle casts. Nowadays, vascular corrosion casts or injection replicae [10] visualize also the microvasculature when studied in the scanning electron microscope (SEM). Moreover, from stereopaired images imported into a PC-based 3D-morphometry system vessel diameters, intervascular and interbranching distances (lengthes), and branching angles can be measured [11-12]. Data allow to test tissues and organs for optimality principles underlaying the design of their blood vascular system [13-18].

Presently, SEM of vascular corrosion casts is used to study the endothelium of blood vessels by the structural details imprinted as negatives on the surface of casts (Fig. 1) Depending on pre-treatment of casted vessels and physico-chemical properties of casting media, e.g. hydrophilicity, hydrophobicity, and replication quality [19-20], endothelial cell borders (ECB) and endothelial cell nuclei (ECN) can be imaged qualitatively. With special treatments ECBs can be highlighted using the backscattered imaging mode of the SEM [21]. Moreover, imprints of flow regulating structures, like endothelial cushions (Fig. 2), sphincters, and venous valves can be identified and localized.

At the level of the individual vessel the technique (i) allows to analyze gross appearance of arterial, venous and capillary vessels and the patterns formed by them (Fig. 3), (ii) localizes and identifies conductive and supplying vessels by their long or short interbranching distances, (iii) enables a detailed analysis of individual microcirculatory units and an estimation of arterial-venous transition distances (Fig. 4), and (iii) displays the characteristic features of sprouting and non-sprouting angiogenesis (= intussusceptive microvascular growth, IMG) with its facets intusssuceptive arborization (IAR), intussusceptive branching remodelling (IBR) and intussuceptive vascular pruning (IPR)[22].

At the network level data on interbranching distances, branching angles and branching geometries which define threedimensional networks and relations of diameters and branching angles of parent vessels to large and small daughter vessels which govern the network hierarchy and its functional abilities can be gained. The quantification of these parameters by 3D-morphometry greatly enlarges our knowledge on circulatory capacities of tissues and organs under normal and pathological conditions, e.g. in tumors [23].

In the presentation we will give examples for the application of SEM of vascular corrosion casts for the analysis of developing (Fig. 5) and fully differentiated (Fig. 6) blood vascular systems under normal conditions whereby the blood vascular system of the model organism Xenopus laevis Daudin and the changes from larval to juvenile and in adult life will serve as a reference.

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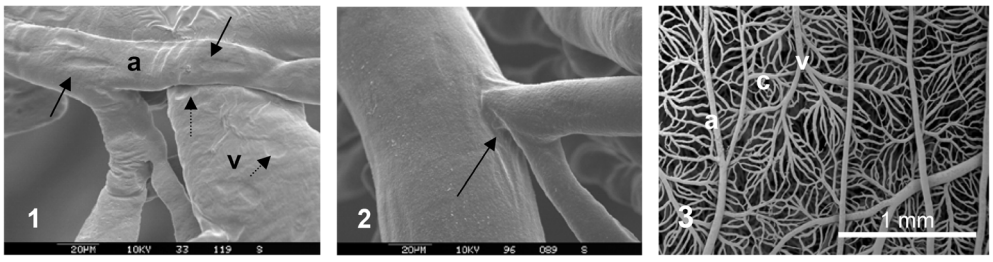

Figure 1. Arterial (arrows) and venous (broken arrows) endothelial cell nuclei imprints on the surface of vascular corrosion casts enable an easy differentiation of arterial (a) and venous (v) vessels. SEM micrograph. **Figure 2.** Intimal cushion (arrow). **Figure 3.** Arterial (a), capillary (c) and venous (v) patterns seen in the large intestine of *Xenopus laevis* Daudin. Serosal view.

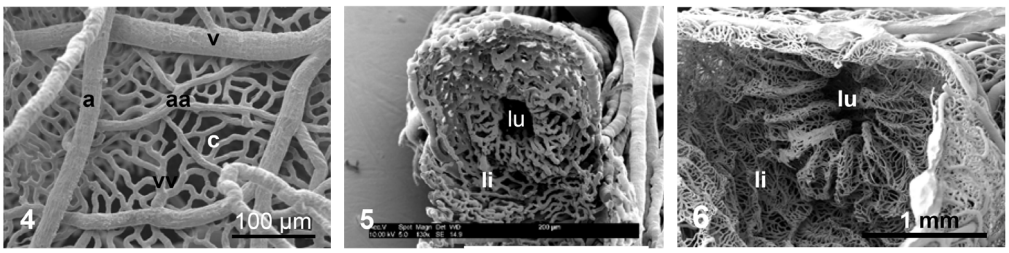

Figure 4. Transition from artery (a) to arteriole (aa), capillary (c), postcapillary venule (vv,) and vein (v). Large intestine. Serosal view. Adult *Xenopus laevis*. **Fig. 5.** Merging of the small intestine (ileum) into the large intestine (li) as seen from the large intestine. lu lumen of the small intestine. Larval *Xenopus laevis* at stage 65. **Figure 6.** Same as Figure 5, but in the adult *Xenopus*. Note the radiating folds coming from the small intestine which soon integrate into the vascular pattern of the large intestine.

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