Scanning electron microscopy and vascular corrosion casting for the characterization of microvascular networks in human and animal tissues

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Vascular corrosion casting is an old method applied by anatomists since a long time (for review and methodical details see [8]). Initially, the viscosity of casting media was very high and thus the technique failed to cast the entire circulatory system from the arterial injection site through small arteries, arterioles, capillaries, venules, small veins and caval veins [9-10]. As casting media soon were improved and thus enabled casting of the entire circulatory system (i) the entire circulatory system of small animals like amphibian tadpoles and (ii) the microvascular network supplying the thick wall of larger blood vessels, the vasa vasorum, e.g. of the human great saphenous vein (HGSV).

Initially the blood vascular system of amphibian tadpoles was studied in Rana [2] using transmission light microscopy and camera-lucida drawings. More recent studies focus on the cardiovascular system of the model organism Xenopus laevis Daudin using intravital video microscopy without [3] or with prior injection of fluorescent dyes [4-5] or multiphoton confocal microscopy on developing blood vessels prior labelled with fluorescent protein, DiI [6] or quantum dots [7]. These in-vivo studies greatly enlarged our knowledge on anlage (vasculogenesis), spatio-temporal growth (angiogenesis), maturation, stabilization, remodelling, and regression of blood vessels under physiological and pathological conditions.

Vascular corrosion casting is an old method applied by anatomists since a long time (for review and methodical details see [8]). Initially, the viscosity of casting media was very high and thus the technique failed to cast the entire circulatory system from the arterial injection site through small arteries, arterioles, capillaries, venules, small veins and caval veins [9-10]. As casting media soon were improved and thus enabled casting of the entire circulatory system [11]. Examination of these vascular casts under the light microscope prevented a thorough analysis with high resolution. Only when in 1971 Murakami [12] used the scanning electron microscope to study vascular corrosion casts a breakthrough was gained. From the many methods enabling imaging of blood vessels here we report on the potentials of scanning electron microscopy (SEM) of vascular corrosion casts (VCCs) in the study of microvascular networks in human and animal tissues whereby we focus specifically on the study of (i) the entire circulatory system of small animals like amphibian tadpoles and (ii) the microvascular network supplying the thick wall of larger blood vessels, the vasa vasorum, e.g. of the human great saphenous vein (HGSV).

The blood vascular system of animals and man is a transport system which supplies oxygen and nutrients to and removes carbon dioxide and metabolites from body cells, tissues and organs, circulates hormones, immune-competent cells and antibodies, and serves the organisms heat exchange with the environment. Under pathological conditions the blood vascular system is both, a route for dissemination of tumour cells (metastasis) and a route for the systemic administration of therapeuticals used in cancer therapies (e.g. chemo-, immuno- and photodynamic laser therapy). Still a better knowledge of the anatomy of the cardiovascular system and its components down to the microvascular level is needed to improve delivery of these therapies.

Presently, many methods are available to image the blood vascular system. Modern imaging methods comprise a broad range of spatial and temporal resolutions (for a review see [1]) and enable to visualize blood vessels both in-vivo and ex-vivo. As spatial resolution of imaging methods is inverse proportional to the size of the area which can be visualized a few techniques remain. They allow a reasonable high spatial resolution and a sufficient large field of view enabling to trace individual vessels over a longer distance connecting two areas far distant to each other though functionally working together as it is the case with e.g. endocrine glands and their target cells, tissues and organs.

From the many methods enabling imaging of blood vessels here we report on the potentials of scanning electron microscopy (SEM) of vascular corrosion casts (VCCs) in the study of microvascular networks in human and animal tissues whereby we focus specifically on the study of (i) the entire circulatory system of small animals like amphibian tadpoles and (ii) the microvascular network supplying the thick wall of larger blood vessels, the vasa vasorum, e.g. of the human great saphenous vein (HGSV).
branching angles could be gained enabling to test real existing microvascular beds in respect to optimality principles underlying the construction of individual vascular beds of tissues and organs in health and disease.

2. Casting the circulatory system of tadpoles of Xenopus laevis Daudin

2.1. Preparation of tadpoles

After staging [15], weighing (mg) and measuring (mm; total length, body length) tadpoles of developmental stages 48 – 66 are killed by immersion in an overdose of an aqueous solution of tricaine methansulfonate (MS 222; 0.03%), and are pinned in supine position on a wax plate submerged in amphibian Ringer solution. After excision of the skin overlaying the heart using microscissors (Fig. 1), the pigmented pericardium is opened, heart and bulbus cordis are exposed, and a ligature (11-0 surgical suture material; diameter: ~25 µm) is placed around the bulbus (Fig. 2). Then the apex of the ventricle is cut open by a small incision with microscissors (Fig. 3) and a thin glass cannula (Figs. 3-4) guided by a micromanipulator is inserted through the ventricle into the bulbus and fixed by the ligature (Fig. 6).

2.2. The injection system

The injection system we use for casting the whole circulatory system of tadpoles of Xenopus (body weight ranges from ~54 mg in stage 48 to ~350 mg in stage 66) is shown in Fig. 7. This injection system has three important advantages, namely (i) it enables to change the syringe used in rinsing the circulatory system with amphibian Ringer solution [16] with that syringe loaded with the ready-to-inject casting medium (in our case: Mercox-Cl(2B) without introducing air bubbles into the injection line when changed for the first syringe (air bubbles also could escape via the opened second outlet of the two-way Y-piece, (b) it enables the injection medium to overcome the long distance from the syringe to the T-connector positioned just before the glass cannula within a short time and – more importantly- without increasing the pressure of the rinsing solution in front of the resin, and (c) it drastically shortens the final distance the injection medium has to overcome before it reaches the bulbus. The time needed to push the resin forward is kept short and - still more important- the injection pressure does not rise within the injection line.
Fig. 1 Tadpole of *Xenopus laevis* Daudin. Stage 53. Ventral view at the cardiac region. The skin over the heart is removed, the heavily pigmented pericard is exposed. **Fig. 2.** Glass cannula used for rinsing and injection of resin. Note the long tapering ending of the cannula. **Fig. 3.** Glass cannula with a drop of amphibian Ringer solution escaping the open tip of the cannula. The drop acts like a lens and enables to control the quality of the broken end of the cannula. Furthermore, the cannula can be controlled to ensure that there are no debris inside the cannula. **Fig. 4.** Heart after removal of the pigmented pericardium. Note the ligature (arrow; surgical suture material 11-0; Ethilon) placed around the bulbus arteriosus. **Fig. 5.** Same as Fig. 4, but the base of the ventricle is incised by microscissors (arrowheads). Note the tip of the glass cannula which is still outside the ventricle (arrow). **Fig. 6.** Glass cannula inserted into the bulbus arteriosus via an incision at the base of the ventricle. Note the tip of the cannula inside the bulbus (arrow). ab..abdomen, at..arterial trunk, ec..external carotid, la..left atrium, r..right atrium, sa..systemic aorta, v..ventricle.
Fig. 7. Setup used for casting the circulatory system of small animals (tadpoles) and excised segments of the human great saphenous veins (HGSV). a. one-way plastic syringe with rubber sealing of plastic plunger and Luer-lock system, b. two-way PVC-connector with the short side connected to the syringe, c. vein flow, d. T-connector, e. short (≤ 10 mm) PVC-tubing, f. glass cannula, g. electrode (cannula) holder, h. micromanipulator, i. PVC-tubing to aluminium bloc, j. syringe pump.

Fig. 8. Aluminium bloc with a horizontal drill hole (arrow) housing the PVC-tubing ascending from the vertical outlet of the T-piece. The device allows to rapidly open and close the injection line when the syringe with Ringer solution has to be changed for the ready-to-inject Mercox. Screw used to occlude the tubing. Fig. 9. Electrode (cannula) holder (h) with glass cannula (gc) and T-piece (T). 1 line from syringe, 2 line to cannula, 3 line to aluminium bloc (ab). M. micromanipulator. Note that the vertical orientation of the third outlet of the T-connector will guide any air bubble into line 3 preventing plugging of blood vessels by air bubbles.

In detail, the injection system consists of (i) a 5-ml one-way plastic syringe with a rubber sealing of the plunger (Fig. 7; a), (ii) a Y-shaped two-way injection line made from PVC tubing (Fig. 7; b), (iii) a vein-flow (size G-21) whose steel needle is cut off (Fig. 7; c), (iv) a small plastic T-connector (Fig. 7; d), (v) a short length of PVC-tubing (Fig. 7; e), (vi) a glass cannula (Fig. 7; f) which is fixed to an electrode holder (Fig. 7; g) mounted to a mechanical micromanipulator (Fig. 7; h), (vii) a sufficient long PVC-tubing connected to the third outlet of the T-connector (Fig. 7; i), and (viii) a device to occlude the latter (Fig. 7; j). This device consists of an aluminium bloc with a horizontal drill hole and a vertical screw which occludes the PVC-tubing passing through the horizontal drill hole when tightly screwed (Fig. 8).

In mounting the cannula to the electrode-holder of the micromanipulator attention must be paid to fix the cannula in a way that the third outlet of the T-connector is in an upright (vertical) position (Fig. 7; k; Fig. 9). This will ensure that
any air-bubbles which might come via the injection line ascend rather into this vertical outlet then into the slightly descending tubing leading to the cannula.

The glass cannulae used are made from borosilicate glass capillaries used by electrophysiologists to pull microelectrodes for intracellular recordings or patch-pipettes. Using a micropipette-puller size and shape of the tip of the cannula can be pulled with constant dimensions (Fig. 2). Before a cannula is inserted into the bulbus its tip is brought into the focus of the stereomicroscope and the tip is ripped off at a site where the diameter best fits the dimension of the aortic valve (Fig. 3). After controlled ripping off the tip of the cannula care has to be taken to avoid sharp edges at the end of the cannula. This can be best controlled when the syringe pump pushes rinsing solution with a flow rate of 1 ml/hour through the cannula making droplets escaping the tip of the cannula (Fig. 3). Fluid droplets function like a lens, enlarge the end of the tip of the cannula and allow for a quick control of the tip edge quality. Once the edge of the tip is considered adjusted to the dimension of the aortic valve of the given tadpole it can be inserted into the bulbus. If the cannula has the appropriate tip diameter it will pass smoothly through the aortic valve without either ripping off the bulbus from the ventricle or perforating the thin wall of the bulbus. Before the ligature is closed around the bulbus one has to assure that the cannula indeed is inside the lumen of the bulbus by focussing onto the tip of the cannula inside the bulbus (Fig. 6). A ligature of 10-0 or 11-0 suture material once closed around the small diametered glass cannula is difficult to re-open and bears a high risk to lesion the bulbus or to rip off the thin tip of the cannula.

2.3. Flow and pressure during rinsing and injection of resin

When the cannula is securely tied inside the bulbus arteriosus the venous sinus and/or the atria are cut open by the microscissors and rinsing with amphibian Ringer solution starts. Considering the low blood volume and the low blood pressure of a tadpole of Xenopus a flow rate of 3-7 ml/hour proofed to be appropriate both for rinsing and resin injection. For the following reasons the perfusion pressure during the injection of casting medium is omitted. First, the (internal) cross sectional area of the tip of the glass cannula is much smaller than any sites along the injection line where a manometer or pressure transducer could be attached, and second, the viscosity of the resin we usually inject ranges from 20-30 centipoise and thus is 5-10 times higher than (human) blood is. According to Hagen-Poiseuille’s law a much higher initial pressure has to be applied to overcome the much steeper decrease in the pressure drop along the injection line. Actually, a much higher than “physiological” pressure has to be applied in vascular corrosion casting if the injection site is far away from the area to be casted as it is the case in casting the entire circulatory system of the tadpole. For practical work we control the amount of ballooning of the bulbus arteriosus and the following arterial trunk and then adjust the injection pressure indirectly by increasing or decreasing the flow rate of the syringe pump. When adjusting the flow rate one has to keep in mind that – depending on the amount of accelerator added and the dilution of the prepolymerized Mercox by monomer – the casting medium remains liquid for a certain period of time only before it starts to polymerize and to increase viscosity exponentially preventing further injection.

2.4. Resin injection

As soon as clear reflux comes from the opened venous sinus and/or the atria the polymerizing resin Mercox CL-2B (Ladd Research Inc., Burlington, Vermont, USA), diluted with monomeric methylmethacrylate (4+1, v+v, 10 ml monomeric methylmethacrylate contain 0.85g initiator paste MA) is injected with a syringe pump at a flow rate of 3-7 ml per hour. A glance through the stereomicroscope at the injection site immediately informs if the cannula is tightly secured in the bulbus. Consequently, there is no leakage of blue resin (Fig. 10). After a few seconds however, resin reflux will start to escape the cut open sites (Fig. 11). Injection is stopped when the effluent resin becomes highly viscous.

2.5. Tempering, maceration, cleaning, drying, mounting and coating

Animals are left untouched for at least 30 minutes at room temperature (20°C) on the wax-plate to allow polymerization of the injected resin. Injected tadpoles are then de-pinned carefully, placed into a water bath (60°C, 12 hours) for tempering, and finally macerated in KOH (7.5%, 12 - 24 hours). When maceration is completed (overnight), vascular casts are transferred using a small spoon or a small vial always covered by the proper fluid via several passages of distilled water into formic acid (5%, 5-10 minutes, 20°C), rinsed again in several passages of distilled water, frozen in distilled water and freeze-dried in a Lyovac GT2 (Leybold Heraeus, Cologne, FRG). Dry specimens are mounted onto specimen stubs using the “conductive bridge method” [17] and sputtered with a thin layer of gold. With this technique “conductive bridges” are fixed under stereomicroscopic control to individual vessels with colloidal silver paste. Thereby, viscous colloidal silver has to be used because too fluid colloidal silver will be sucked up into capillary meshes masking individual vessels. Furthermore, interesting details of the specimens should not be hidden by the attached “conductive bridges".
2.6. SEM inspection

Coated specimens are investigated with a scanning electron microscope (e.g. Stereoscan 250, Cambridge Ltd., Cambridge, UK; Philips ESEM XL-30, FEI, Eindhoven, NL) at an accelerating voltage of 5-10 kV. This accelerating voltage is high enough to investigate all those details at the (luminal) surface of casted vessels which give useful information (e.g. cell nucli imprints). Accelerating voltages higher than 10 kV will cause a higher thermal burden to the specimen with the risk of specimen damage. The higher spatial resolution gained when the SEM is operated with higher accelerating voltage it will display the limits of the replication quality of the injection resins and it will reveal even the proper structure of the resin used if for example a high resolution scanning electron microscope (HRSEM) is used to inspect vascular corrosion casts.

2.7. Remounting specimens

After a first documentation and analysis of superficial (external) vascular layers specimens are often removed from specimen stubs by ripping off the “conductive (metal) bridges”. Great care has to be taken not to break off any casted vessel. After removal coated vascular casts are immerged into fresh distilled water. Generally, cast specimens will float on the surface of the distilled water. To submerge specimens the vial with the floating cast is placed into a desiccator and evacuated. Several cycles of evacuation followed by ventilation with ambient air will be needed until the specimen sinks and becomes submerged in bidistilled water. Evacuation of the floating specimens has to be done cautiously as application of too high vacuum leads to the formation of large air-bubbles which in turn may destroy the specimen when leaving it. Addition of a small amount of ethanol to the distilled water also facilitates submersion of casts. Before re-freezing the specimen the side where landmarks allow a controlled sectioning with a mini-wheel-saw has to be faced towards the bottom of the vial. After removal of the ice-bloc with the enclosed specimen from the vial the ice-bloc will be frozen upside-down to a wooden support plate fitting the cutting device.

Fig. 10. Injection of the blue resin Mercox-Cl-2B. Note the slight bulging of the bulbus (ba)) indicating that the initial portions of the systemic aortae (1), of the branchial arteries (2) and of the pulmonary arteries (not to be seen) are intact. Note also the strong pigmentation of the arterial trunks (arrows).

Fig. 11. Resin reflux (rr) from the opened venous sinus and atria. Note the transversal muscle (tm).

2.8. Sectioning ice-embedded vascular corrosion casts

To enable correlation with paraplast embedded tissue sections ice-embedded corrosion casts are frozen orientated onto wooden specimen holders and sectioned transversely, longitudinally, tangentially or horizontally with a mini-wheel-saw placed in the chamber of a cryo-microtome. Sectioned ice-embedded specimens then thaw in distilled water, get cleaned and are refrozen in bidistilled water for subsequent freeze-drying in a Lyovac GT. Further steps in specimen preparation are as described above.

According to the needs specimens are repeatedly sectioned to expose interesting vascular territories and to pinpoint the course of individual vessels of interest. To expose individual vessels over longer distances overlaying vessels can be ripped off manually under stereomicroscopic control using sharply polished insect pins (size: 000) or minute needles. As any ripped-off vessels will be lost it is adviseable to document every preparatory step in the SEM before the next layer of vessels is ripped off.
3. Casting the vasa vasorum of the human great saphenous vein (HGSV)

3.1. Specimens

Three to six centimeter long segments of middle or distal human great saphenous veins from patients (4 females aged 69–80 years, average: 75 years; 9 males aged 60–79 years, average: 71 years) undergoing aorto-coronary bypass surgery are studied. Segments are stored in ice-cooled heparinized saline (1000 I.U./ml 0.9 saline) and were casted within 6 – 10 hours after harvesting.

3.2. Preparation

HGSV segments are submerged in heparinized saline (20°C), pinned with their ends to the wax bottom using two insect pins, and an arterial feeder of the vasa vasorum is searched. Although we know from human cadavers injected with India ink that in-situ arterial feeders invade the adventitia of the HGSV every 0.5 - 1.5 cm and that arterial feeder and venous drainer run closely aside each other in most explanted segments it was difficult to find and to correctly identify an arterial feeder large enough to be successfully cannulated. While draining veins in most cases are easily to find their arterial partners tend to retract and thus are difficult to find. Once found the arterial feeder is dissected over a short distance and a ligature from suture material sized 10-0 or 11-0 (Ethilon) is placed around.

3.3. Cannulation, rinsing and resin injection

Cannulation of the arterial feeder is done with a micromanipulator. Due to the small diameter of the arterial feeders of the vasa vasorum (≤100 µm) the tip of the cannula has to be very small. To enable a clear view at the site where the cannula can be introduced into the arterial feeder we use a 40x magnification. This has the adverse effect that the depth of focus is very low and the tip of the cannula which has to be guided with a rather oblique angle towards the arterial feeder will be in focus only over a very limited range. As cut arterial vessels tend to occlude their lumen by contracting their wall towards the lumen only a cannula with a tip-diameter much smaller than the diameter of the arterial feeder will glide easily into the vessel and allow to be pushed forward inside the vessel to finally be securely tied in place by the ligature. On the other hand the smaller the lumen of the glass cannula is the higher is the pressure needed to inject the casting medium and the greater is the risk that the polymerizing resin with its increasing viscosity cannot be injected sufficiently long to cast larger areas of the vasa vasorum vascular bed within the vessel wall. Ideally, the lumen of the cannula should be as large as possible for any given outer diameter of a cannula which in turn has to be seen in relation to the size of the lumen of the blood vessel it has to be introduced into.

When the cannula is securely ligatured in the arterial feeder rinsing with saline is started (Fig. 12). A first indication that actually an arterial feeder is cannulated is gained if blood escapes from the closeby venous drainer and the wall of the HGSV gets whitish. A retrograde injection via the much larger venous drainer does not work and injected resin will stop very soon or – if higher pressure is applied – it will cause rupture of the vessel.

The injection of the liquid resin (Mercox-Cl-2B) is done by a perfusor (syringe) pump with a flow rate of 3-7 ml/hour (Fig.13). After hardening of the injected resin specimens are processed the same way as reported above for tadpoles under 2.1. For denomination of consecutive branching levels of vasa vasorum the terminology proposed by Schönenerger and Müller (1960) is used.

4. Results

4.1. The circulatory system of tadpoles

Injection of the resin via the bulbus arteriosus enables the filling of the arterial trunks and in turn filling of the three major vessels leaving bilaterally the arterial trunks, i.e. (from anterior to posterior) a) the systemic aortae, (ii) the branchial arteries, and (iii) the pulmonary arteries. Although resin is injected through all three main arteries and sufficient resin efflux from the opened atria or the opened venous sinus occured there was no single tadpole whose entire blood vascular system was cast completely. Instead, tissues and organs in general reveal a varying degree of filling. Few organs only are completely filled with resin while others are only partially filled or are not casted at all. The organs which are casted in most injections are those which are strongly perfused, i.e. gills, liver, kidneys, digestive tract and lungs. Following, two selected organs and their microvascular patterns, namely gill filter plates and selected portions of the digestive system (esophagus and stomach) are described.
Fig. 12. Cannulating the arterial feeder of the vasa vasorum of the human great saphenous vein (HGSV). Note the glass cannula (1) inside the artery. An insect pin (2) tows the artery towards lateral and thus helps to keep the cannula in place. To visualize the tiny artery a piece of negative film (3) is positioned beneath the artery. Fig. 13. Injecting Mercox-Cl-2B into the arterial feeder. Note the resin (arrows) leaking from branches of the feeder which have been opened when the segment of the HGSV has been harvested during surgery.

4.1.1. Gill filters

The filter apparatus of tadpoles consists of four filterplates (I-IV) on each side of the branchial chamber. Filter plates have parallel arranged filter rows which are heavily vascularized (Fig. 14). The filter apparatus is supplied bilaterally (from anterior to posterior) by a branch of (i) the thyro-pharyngeal artery, (ii) the branchial artery, and (iii) a branch of the pulmonary artery. Feeding arteries branch and run across the filter plates giving off terminal arteries which feed the filter row vessels from beneath (Figs. 15 and 16). Terminal arterioles capillarize and postcapillary venules merge several times within quaternal, tertiary, secondary and main filter veins, which leave the filter rows to form the draining filter plate veins. Filter plate veins drain via the internal jugular vein into the left atrium. As gill filters are tadpole-specific organs which regress during metamorphosis the highly complex three-dimensional network of the filter rows regresses during metamorphosis whereby the vascular patterns gradually become less and less complex until finally all vessels have regressed.

Fig. 14. Microvascular patterns of two filter rows (FR) of a tadpole in developmental stage 57 as seen from the buccal cavity. 1..main fold vein of filter row. Arrows mark direction of blood flow. Fig. 15. Arterial supply of three filter rows (FR) as seen from beneath the filter rows. Note the transverse running segment of a filter plate artery (1) abutting branches (2) which run beneath the filter rows (FR) giving rise to terminal arterioles (3) which supply the filter fold vessels (4). Arrowheads in Figs. 15 and 16 mark the transition from the capillary side to the venous side. Fig. 16. Terminal arterioles (3) capillarize and supply the quaternal folds of the filter rows (arrows).

4.1.2. Digestive tract

The digestive tract of tadpoles changes dramatically during metamorphosis when the previous herbivorous tadpole metamorphoses and becomes a carnivorous juvenile. So the intestine looses 78% of its initial length and the initially single-layered, immature looking vascular bed of the intestine gradually becomes a three-dimensional network, which forms longitudinally running intestinal folds by means of intussusceptive microvascular growth. The vascular bed of the glandular stomach (manicotto), which in early metamorphosis reveals wide, immature vessels (Fig. 17), which embrace the gastric glands (Fig. 18) mature within the stomach wall at the height of metamorphosis and thus, arteries and veins can be clearly differentiated by their gross morphology and branching/merging patterns (Fig. 19).
5. Discussion

5.1. Technique

Since its introduction by Murakami in 1971 [12] the technique has been widely used to study the microvascularization of a wide series of tissues and organs during embryonal, fetal, larval, juvenile and adult and old age both in normal and pathological conditions (for reviews see [19-26]). From a series of technical papers we know much about the physico-chemical properties of resins used in vascular casting [27-29] whereby for most applications (i) replication quality [27], (ii) linear and volume shrinkage and (iii) thermal stability of the resin used are the most important parameters. This is because the former defines which details of the luminal surface of casted vessels can be studied [27], while the latter become critical if linear and angular measurements are made by 3D-morphometry [13,14,28].

In respect to the application of the technique in the study of the luminal surface of the blood vessels we still know too little about the influences which the commonly used casting media methylmethacrylate [12, 27, 29, 30], Batson #17 [31], latex [32], Mercox [27,29] and polyurethane [33] have on vascular endothelia. At the level of the vascular segment we have a good knowledge of the different forms casted vascular segments may reveal and which of them are real vascular structures and which are artifacts. This topic is most important if vascular sprouts, non-sprouting angiogenesis (= intussuscipetive microvascular growth) and its various facets like intussusceptive arborisation (IAR) and intussusceptive pruning (IPR) [34-36] or regressing vascular networks [37-39] are in the center of a study. Finally, miniaturization of the cannulae used for injections, the use of micromanipulators together with the use of extremely thin (18-25 μm), but flexible suture materials for ligaturing the cannulae into individual blood vessels, nowadays enable to inject casting media even into arterial feeders of vasa vasorum [40].
5.2. Results

Presently, scanning electron microscopy of vascular corrosion casts allows to study the blood vascular system at the level of (i) the endothelium, (ii) the vessel segment, and (iii) the network.

At the level of the endothelium characteristic imprints can be found. These imprints result from (i) the high endothelium of veins in lymphatic organs [41] and from (ii) endothelial cell nuclei which are longish to oval and orientated parallel to the long axis in arterial vessels and round in venous vessels [42]. Furthermore, using a silver impregnation technique and investigating the surface of vascular casts with the scanning electron microscope which is operated in the backscattering mode cell border lines of endothelial cells can clearly be seen [43-44]. Due to the limits of the replication quality of available casting media endothelial fenestrations cannot be replicated [27].

At the level of the vascular segment imprints of flow regulating devices like flow dividers, intimal cushions, sphincters and venous valves can be localized and studied qualitatively and quantitatively. Furthermore, diameters and lengths of vascular segments, e.g. interbranching distances, can be measured and data gained can be used to test vascular segments for optimality principles underlaying their design [45-46].

At the level of the network intervacular and interbranching distances and branching angles between parent vessel and daughter vessels which define the specificity of individual vascular networks can be measured and thus allow testing optimality principles of entire networks. The question if tissue and/or organ specific vascular patterns exist [47] is yet still not fully answered.

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References


