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Scanning Electron Microscopy of Vascular Corrosion Casts in Biological and Biomedical Research

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Abstract

The cardiovascular system is the first system to develop and to function. It supplies tissues and organs with oxygen, nutrients, hormones, immune competent cells and others and deliberates them from waste products and metabolic heat. Many attempts were made to gain insights into its three-dimensional structure by injecting air, liquids, waxes or hardening substances.

Keywords

Hormones • Arteries • Veins • Blood vascular system • Capillaries

About the Study

A real breakthrough was gained some 50 years ago [1-5]. Casting media became available which replicated the entire blood vascular system from the arteries through capillaries to the veins and resulting vascular casts were robust enough to allow their examination under the scanning electron microscope [6-13]. These resins resist corrosive agents used to remove soft and hard tissues and replicate and preserve minute details of the luminal (endothelial) surface of blood vessels which in turn enable to distinguish between arterial and venous vessels by the characteristic shapes and orientations of endothelial cell nuclei imprints presented at the casted surfaces [14-20] (Figure 1). For more details, see the references [21-29].

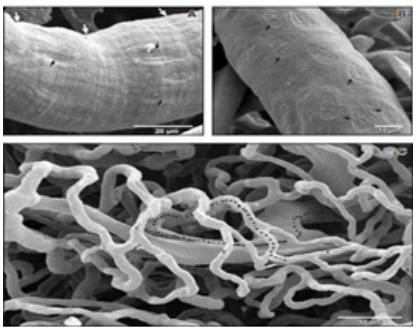

Figure 1. Characteristic endothelial cell nuclei imprint patterns displayed on the surface of vascular casts. Scanning electron micrographs: A.Arteriole with longish imprints of endothelial cell nuclei orientated parallel to the longitudinal axis of the artery (thin arrows). Thick arrows point at imprints of single or groups of vascular smooth muscle cells (sphincters),B.Vein with shallow ovoid to roundish imprints of endothelial cell nuclei (arrows), C.Capillaries (c) building a three-dimensional meshwork. Dotted line indicates a capillary-venous transition route.

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Literature Review

Modern casting media are cuttable into slices by razor blades can be frozen in distilled water and thereafter sectioned by a mini wheel saw or they can be micro-dissected using fine tipped insect pins to expose and re-examine individual vascular territories layer by layer in consecutive SEM sessions [12,30].

For studies of vascular patterns, qualitative data is often enough. Quantitative data on vessel diameters, inter-branching and inter-vascular distances as well as branching angles are needed for hemodynamic calculations of interesting vascular territories. This data can be gained by 3D-morphometry of stereo paired scanning electron micrographs [31-35]. Data gained allows insights into hemodynamic properties of individual vascular segments like wall shear stress [36]. Moreover, these data enable to test real vascular networks for optimality principles [37,39].

In biological research blood vascular systems of individual organs or tissues can be studied on a phylogenetic or an evolutionary scale [40-46]. In these studies, similarities/dissimilarities in origins, courses, branching patterns and areas of supply or drainage of individual vessels are in focus aiming to understand how the blood vascular system maintains blood supply under altered needs according to functional changes of individual tissues and organs. Beyond this, SEM of Vascular Corrosion Castings (VCCs) elegantly allows to locate flow regulating structures, such as muscular sphincters, flow dividers, intimal cushions, and venous valves [47-51] (Figure 2). Furthermore, one can also study Arterio-Arterial Anastomoses (AAAs), Arterio-Venous Anastomoses (AVAs), and Veno-Venous Anastomoses (VVAs).

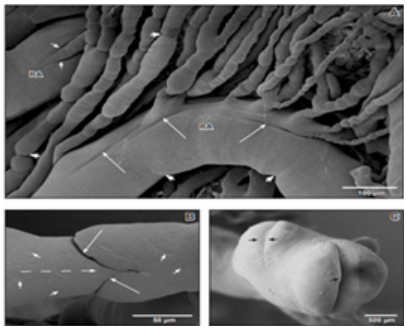

Figure 2. A.Renal Arteries (RA) giving off numerous afferent glomerular arterioles. Note imprints of an intra-arterial cushion (short thin arrows), flow dividers (long thin arrows), and vascular smooth muscle cells (sphincters) (short thick arrows), B.Venous valve (long thin arrows). Short thin arrows point at imprints of endothelial cell nuclei. Dashed line indicates the direction of blood flow, C.Two venous valves (arrows) where the retrograde flow of resin was stopped by the valves.

SEM of VCCs is the method of choice in the study of venous portal circulations, where postcapillary venules form portal veins, course over shorter or longer distances, and then capillarize again. Such portal circulations studied in vertebrates are the hypothalamo-hypophysial portal system in the brain, the hepatic portal vein system, the renal portal vein system and the pancreas insulo-acinar portal system [21,22,52-59].

The embryonal and early larval development of the cardiovascular system is excellently visualized by confocal micro-angiography. This technique is well suited for optically clear (transparent) thin animals but fail in opaque and thick objects. Here SEM of VCCs can be applied. Our group focusses upon spatio-temporal aspects of growth and regression of blood vessels in the Xenopus laevis model organism. Xenopus is an anuran amphibian and undergoes drastic changes in basically every organ/tissue during metamorphosis [60]. Most obvious is the loss of larval-specific organs, like the gills and tail. These organs are highly vascularized in early stages of metamorphosis where the growth of blood vessels dominates [61,62]. At the height of metamorphosis (climax), gills and tail are resorbed and regression of a highly differentiated, complex vascular system can be studied [63,64]. But also the microvascular anatomies of chick embryos, mouse embryos, rat embryos and of isolated human fetal organs have been successfully studied by this technique [65-72]. Angiogenesis research is another field of application of SEM of VCCs. Here blood vessels that undergo sprouting and/or non-sprouting angiogenesis can be identified and localized (Figure 3).

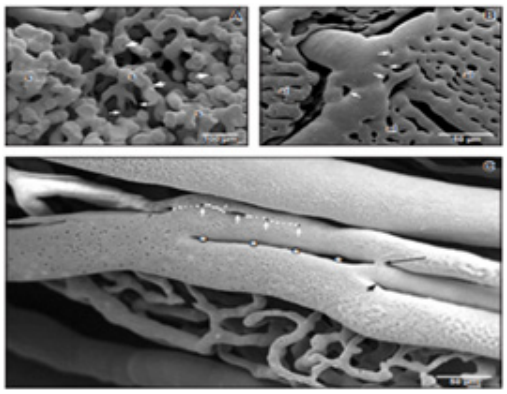

Figure 3. Signs of sprouting and non-sprouting angiogenesis (intussusceptive microvascular growth) as displayed by vascular corrosion casts:A.Sprouting angiogenesis. Thin arrows point at vascular sprouts, thick arrows at sites of intussusceptive angiogenesis. s sinusoids, B.Non-sprouting angiogenesis. Intussusceptive branch remodeling (arrows). al alveolus, C.Non-sprouting angiogenesis. Intussusceptive branch remodeling (thin short arrows) Dotted line indicates the possible shift of the future origin of the vessel towards the right. Short thick arrow points at a site where intussusceptive pruning takes place. Note anastomosis (thin long arrow) between two vessels. Asterisks mark the site of a tissue lamella separating adjacent vessels.

In corrosion casts, vascular sprouts impose as blind ending tapering vessels preferentially occurring at capillary and postcapillary venular sites. Their identification in vascular casts should always be related to the state of the tissue under observation (healthy vs. diseased; growing vs. fully differentiated vs. involuting). Non-sprouting angiogenesis (Intussusceptive Microvascular Growth, IMG) and its facets Intussusceptive Arborization (IA), Intussusceptive Branch Remodeling (IBR), and Intussusceptive Pruning (IP) can be identified. Signs of non-sprouting angiogenesis impose in vascular casts as shallow to deep, round, oval or longish impressions or as holes or slits of different sizes and shapes [73-80].

In biomedical research SEM of VCCs is applied in atherosclerosis research, diabetes research, nephrology research, ophthalmologic research, tissue engineering research and tumor research [81-101]. Studies on tumor vascular casts show that the normal hierarchy of the blood vascular system can be highly disturbed and vascular patterns can extremely differ. In tumors, the positive identification of casted structures such as blood vessels is sometimes difficult since casts of tumor vascular beds differ greatly in their appearance from casted normal blood vessels. Within short distances they change their diameters, kink, out pouch, constrict, or end abruptly. In areas of vascular mimicry, imprints of tumor cells can be found on their surfaces and in necrotic areas casted structures are found that resemble extravasations. A clear differentiation of casted vascular structures from artifacts is difficult and needs supplemental techniques.

Like other techniques, vascular corrosion casting is also prone to artifacts. Incompletely casted blood vessels impose as blindly ending vessels with rounded tips. They can be positively differentiated from broken vessels, which show straight, sharp endings, and also from sprouting vessels, which impose with gradually tapering endings. In some cases, “plastic strips” are found around vascular casts. According to their shape and rather annular structure, they are considered to represent plastified vascular smooth muscle cells or pericytes [102-107].

Discussion and Conclusion

Vascular corrosion casts are increasingly investigated by micro-computer tomography (μ-CT). To gain spatial resolution comparable to that of the conventional SEM, only very small specimens can be studied, a disadvantage if vascular routes connecting areas far apart each other are in the focus of interest.

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