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EXAMINATION OF INJECTED SPECIMENS BY CONFOCAL LASER SCANNING MICROSCOPY AND SCANNING ELECTRON MICROSCOPY

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Abstract

Although corrosion casts commonly examined by scanning electron microscopy (SEM) offer good insights into the general arrangement of the microvasculature of certain organs, no information can be obtained from such specimens on special morphological data or dynamic events. Thus, most investigators are obliged to make their interpretations of cast structures only on the basis of indirect criteria. A synopsis, which considers the most important data in that respect, is given for the blood and lymphatic microvessels. Moreover, a methodological approach is introduced, based on the application of fluorescent resin, which allows the examination of cast structures in uncorroded specimens together with the surrounding tissue by confocal laser scanning microscopy (CLSM). When two differently stained resins are injected at different sites, e.g., Mercox-fluorescent yellow into an artery and Mercox-rhodamine into the interstice, blood vessels and lymphatics can be well distinguished. In lymph nodes, the system of lymphoid spaces and that of intranodal sinuses can be separately represented with the same technique. Applications and advantages of the CLSM method, which is a useful tool for an extended interpretation of corrosion casts in SEM, are shown in cast specimens from tongue, skin, and liver in rats. In this study, both corroded and uncorroded specimens were considered. In addition, a new approach is demonstrated which combines casting technique and fluorescence microscopy with investigations carried out under experimental conditions. This allows the examination of microvascular casts together with labelled cells in liver and spleen in a state of phagocytosis.

Key Words: Laser scanning microscopy, fluorescent resin, corrosion casting, tissue specimens, scanning electron microscopy.

Introduction

A well-known step of the procedure of corrosion casting for scanning electron microscopy (SEM) is the maceration of soft tissue. This step removes all organic components, while the polymerized resin withstands the corrosive forces of the concentrated lyes and acids applied. Although the specimens obtained, e.g., microcorrosion casts of blood and lymphatic vessels, offer insight into the systemic nature of the vasculature of a certain organ, a clear assessment of morphological details by this technique is still limited. The knowledge of the structural organization of the tissue originally surrounding the luminal casts of vessels remains poor and refers only to indirect features appearing at cast surfaces. Hence, a good quality of the cast specimens is an inevitable precondition for each morphological evaluation.

Among the numerous factors influencing the quality of corrosion casts in microvascular research, some are connected to the physico-chemical properties of the medium and the mode of application (injection pressure, site of injection) [4, 5]. Others concern the procedure of cleaning and sputtering to which the specimens have been submitted. All these factors, however, cannot be considered in the present paper (for more information, see reference [4]). In the present paper, we examine (1) to what extent do the vascular casts reflect the true morphological conditions, and (2) how can conventional SEM of corrosion casts be supplemented by parallel confocal laser scanning microscopy (CLSM) of fluorescent specimens.

It is evident that positive identification of single microvessels and, perhaps, assessment of a functional situation from corrosion casts, are only possible when a complete filling of the vessels has been achieved and artifacts, caused either by incomplete casting or overfilling, have been avoided. On the other hand, imprint patterns, created by the endothelium (cellular outlines, nuclear imprints) [15] and by subendothelial structures (myocytes, pericytes) [1, 2, 3], occurring on cast surfaces, facilitate the classification of microvessels. The

Table 1. Identification of vascular casts in SEM according to indirect criteria.

	endothelial imprints caused by				
	cellular outlines	nuclei	branching pattern	dimensions	special features
arterial vessel	slender, elm-shaped	longish	organ specific	smaller than accompanying veins	wrinkled lumenal profile, spirally or circularly arranged imprints of myocytes
capillaries	lozenge-shaped	roundish	organ specific network	constant small diameters	bottleneck-like constructions at origin (sphincter)
sinusoids (e.g., liver, spleen)	irregularly shaped	roundish	organ specific pattern	wider than capillaries	opening to tissue
postcapillary venules (lymphoid tissue)	irregularly shaped	roundish	solitary structures; little branched	wide calibers	irregular lumenal profile, imprints of sticking lymphocytes (recirculation)
venous vessels	broad lozenge-shaped	roundish, oval	organ specific ramification	larger than accompanying arteries	valves
initial lymphatics	broad oak-leaf-shaped open junction system	oval	plexus-like arrangement	wide and strongly varying calibers	sporadically occurring valves, outpocketing structures
collecting lymphatics	similar to veins	roundish, oval	parallel running converging system	varying calibers with beaded appearance (lymph angions)	chain-like arrangement of valves

peculiar structures described as "plastic strips" [5, 6, 14], frequently found around the luminal casts of arteriolar vessels and blood capillaries, provide additional information which may contribute to a better determination of blood microvessels at the transition of arterioles to capillaries. Other features, such as the type of branching, the dimensions of vascular casts and the occurrence of special structures like sphincters [1, 16] help to distinguish capillary vessels of the terminal blood vascular system. Structural features and parameters, which characterize blood and lymphatic microvessels in microvascular research, are summarized in Table 1.

Unfortunately, many corroded cast specimens do not reveal sufficient data which, in analytical morphological studies, are needed for a clear interpretation of individual structures. Thus, a fundamental knowledge of the elements forming the vascular wall and of the structural relationship between the vessel and the surrounding tissue is required. Moreover, in some cases, true lymphatics must be distinguished from prelymphatic channels and simple tissue spaces. Occasionally, a clear decision between artificial and dynamic phenomena, both expressed by cast structures, is also necessary.

To obtain additional information on these details, we designed a technique based on the application of fluorescent resin and the examination of uncorroded tissue under the CLSM. This study shows the application and efficiency of that method performed prior to maceration and subsequent SEM examination.

Material and Methods

Twelve Wistar rats of both sexes (weighing 240-250 g) were used in this study. The animals were anesthetized with ether, followed by Trapanal® (Byk Gulden, Konstanz, Germany) intraperitoneally and, after the

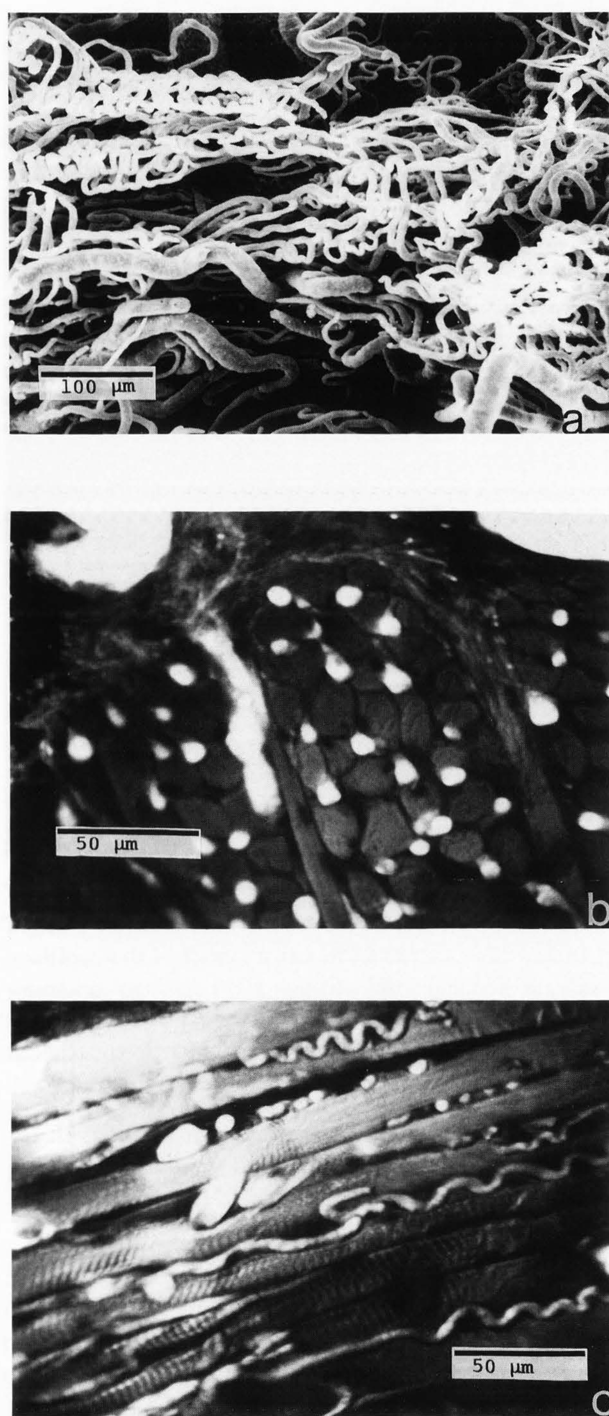

Figure 1. (**a**) Scanning electron micrograph of a corrosion cast of the skeletal muscle of the rat tongue. The blood capillary system is represented here without the muscular elements they are related to. (**b** and **c**) In these CLSM images of a fluorescent cast tissue specimen taken in (**b**) cross- and (**c**) longitudinal-sectional areas, the topographic relations between single capillary casts and individual muscle fibers become obvious.

abdomen and thorax were opened, they were killed by a cut through the heart. The blood vascular system was rinsed with Ringer solution, to which 0.2 ml heparin (Hoffmann La Roche, Grenzach, Germany) was added, via a cannula placed in the ascending aorta. Ten milliliter undiluted Mercox® (Vilene Co., Tokyo, Japan) mixed with 1 g catalyst was used as resin for casting of the blood and lymphatic vessels and interstitial spaces.

The resin was stained with the fluorochromes rhodamine (E. Merck, Darmstadt, Germany) and fluorescent yellow (Fluka, Neu-Ulm, Germany). For this purpose, 200 mg of the dye was added to 20 ml of the monomeric phase while whirling it on a rotary shaker. After mixing with catalyst, the stained resin was injected into the aorta, using hand pressure, for casting of the blood vascular system and into the interstice of the rat tongue for the representation of the tissue space system and the initial lymphatics. Mercox® was also placed into the lymph nodes of the neck and other body regions in order to fill the nodal lymphoid tissue space system. In other experiments, stained Mercox was applied at both sites simultaneously in order to cast arterial or venous vessels, blood vessels and lymphatics, or vascular structures and tissue spaces. The animals were left at room temperature for one hour. Thereafter, organs like tongue, liver and spleen, and lymph nodes of the neck region and those of the para-aortic group, were dissected and fixed by immersion in 2.5% glutaraldehyde. After incubation at 40°C for one night, one half of each organ was macerated in 35% potash lye and further processed for SEM as described earlier [9, 10]. The other half of the organ was cut by hand into thin sections with a razor blade. The sections were placed on slides, quickly (i.e., without allowing the specimens to dry) covered by a cover glass and sealed with Mercoglas® (Merck). These specimens were examined with a CLSM (E. Leitz, Bensheim, Germany), while the corrosion casts were subjected to the SEM examination on the AMR (E. Leitz) scanning electron microscope. For fine structural analysis, a field emission SEM (Hitachi S 600) was used. Accelerating voltages in the range of 5 to 25 kV were used.

In a special group of experiments carried out in living rats, the animals were injected intravenously and into the interstice of the tongue, skin and lymph node with fluorescent microbeads (Fluoresbrite®, Polysciences Europe GmbH, Eppelheim, Germany). This was done to allow macrophages of the connective tissue, liver and other organs to take up these particles, and thus, to indicate zones of phagocytotic activity prior to the casting procedure with fluorescent resin. The injection was performed with 0.2-0.5 ml of the commercially available solution, depending upon the size of the specimen.

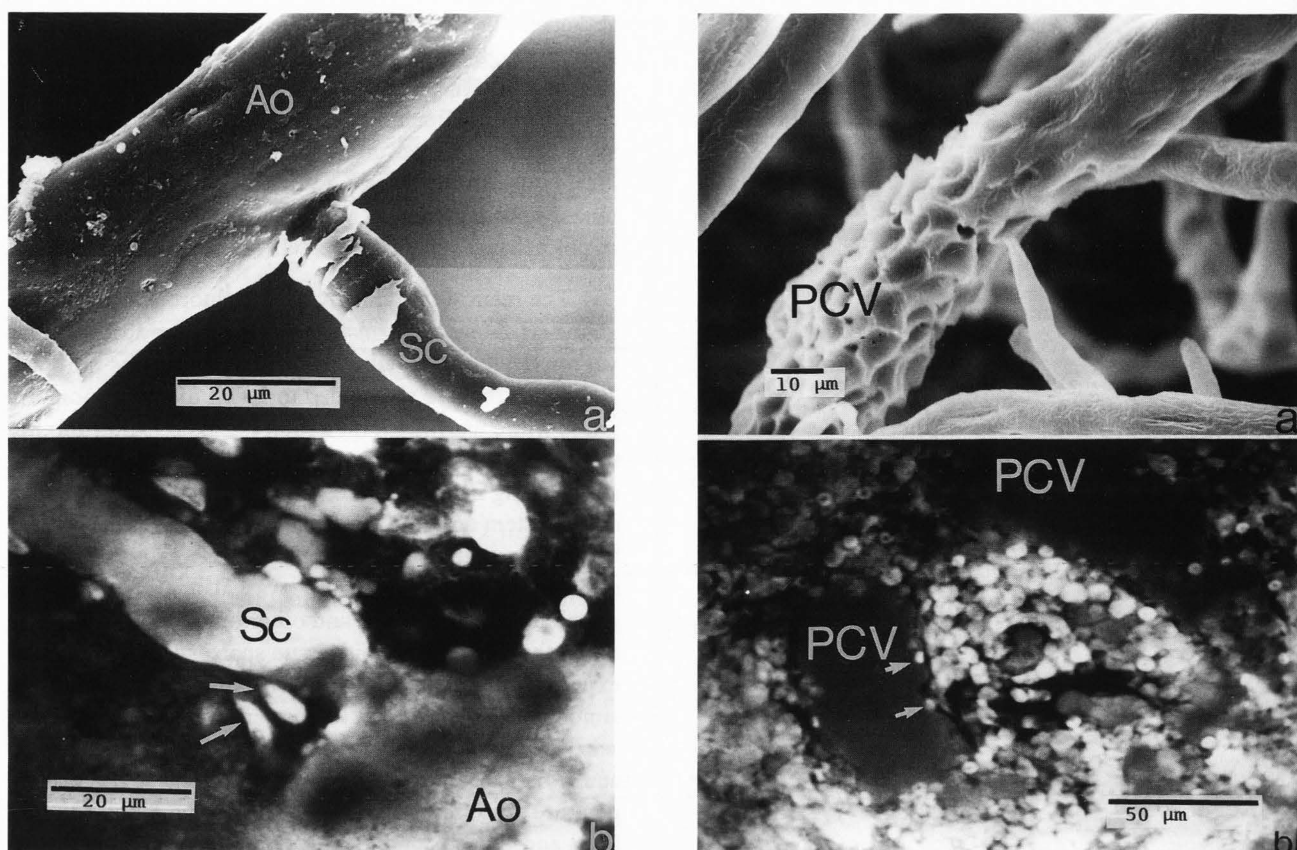

Figure 2 (at left). (**a**) Scanning electron micrograph of a corrosion cast with a sphincter-like constriction of a blood capillary (Sc), that branches off from an arteriole (Ao) in the rat brain. The narrowed initial segment of the capillary is wrapped in circular "plastic strips" mimicking single smooth muscle elements. (**b**) In this confocal laser scanning micrograph of an uncorroded cast specimen of the rat brain a sphincter-like constriction of a blood capillary sphincter (Sc) is recognizable as well. The image taken in the optical plane through the sphincter zone shows the true endothelial and smooth muscle cells (arrows) forming the cytological base for the sphincter mechanism. Ao: arteriole.

Figure 3 (at right). (**a**) The scanning electron micrograph of a corrosion cast of a rat lymph node from the neck region reveals the cast of a postcapillary venule (PCV) with its characteristic irregular surface profile created by bulging endothelial cells and homing lymphocytes. (**b**) Corresponding image of two resin-filled postcapillary venules (PCV) of an uncorroded tissue specimen obtained by CLSM. The luminal cast of that vessel is surrounded by prominent endothelial cells and numerous lymph cells, some being in a state of transmural diapedesis (arrows). Thus, the cytological conditions prevailing around the vascular cast zone can be easily studied and directly connected to the peculiar imprint pattern of that vessel seen in corroded specimens by SEM.

Results

CLSM of cast tissue

In cast tissue specimens, ducts and spaces filled with resin appear in CLSM as bright fluorescent structures, and are easily distinguishable from other tissue components which exhibit either only weak autofluorescence or are stained with common dyes. Thus, single vascular casts can be accurately studied in relation to their topographic position in the stromal tissue or parenchyma within an organ. In skeletal muscle, for example, the blood capillaries can be studied in relationship to the single muscle fibers which they supply (Fig. 1). In uncorroded casts, tissue spaces, prelymphatic channels, and initial lymphatics can also be examined in relationship to the surrounding tissue when fluorescent resin is injected into the interstice. From those preparations, quantitative evaluation of the vascular supply of the parenchyma is also possible, if the ratio between the cells of the parenchyma and capillaries is calculated within a certain tissue area.

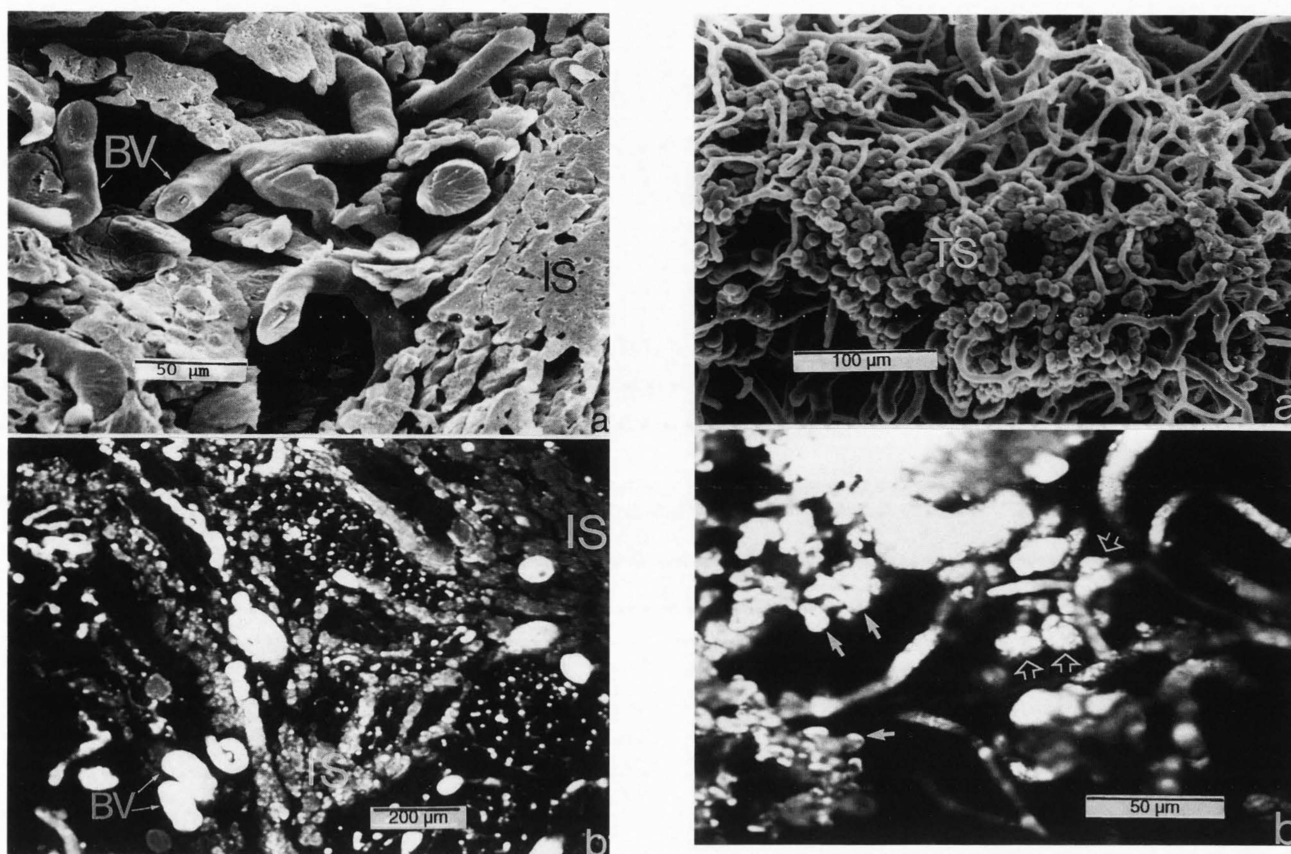

Figure 4 (at left). (**a**) Scanning electron micrograph of a double-filled corrosion cast of the rat tongue. The resin was injected into the interstice, thus casting the interstitial spaces (IS). Simultaneously, resin was injected into the aorta by which procedure the blood vessels (BV) of the tongue were cast, too. (**b**) Uncorroded specimen of the skin (rat upper lip), similarly treated with two differently stained resins, as observed in the CLSM. The interstitial space system (IS) appears in the original CLSM color micrograph as a reddish structure and the blood vessels (BV) as yellow-green. Both the interstitial spaces and the blood vessels become easily distinguishable and can be delimited from other components of the tissue.

Figure 5 (at right). (**a**) Scanning electron micrograph showing a cast lymph node. The specimen was perfused both interstitially and via an artery. The intricate system of lymphoid tissue spaces (TS) and the blood vascular system are shown. (**b**) Confocal laser scanning micrograph of a similar corroded specimen of a lymph node injected with two differently stained resins. The cast tissue spaces appearing in the left part of the image (solid arrows) are red stained in the original colored image, while the casts of the blood vessels with accompanying bead-like structures in the right part of the image (hollow arrow) are green-yellow stained. The bead-like structures can be identified as extravasates of the blood vessels in that area because they exhibit the same color features in the CLSM as the related blood vessels.

On the other hand, the morphology of the vascular wall surrounding the luminal cast of a vessel also becomes obvious in uncorroded specimens studied by CLSM. The special structural properties of the vascular wall characterizing the sphincter zone of some blood capillaries can be made directly visible by optical sectioning in the CLSM (Fig. 2). In that way, the relation between the endothelium and subendothelial structures and the correlated pattern of imprints and plastic strips appearing on that cast segment can be studied (more information on these particular phenomena can be found in reference [12]). The special structural differentiation of the postcapillary venules in lymphoid tissue, reflecting the phenomenon of lymphocyte homing, may serve as a further example to compare the peculiar appearance of these vessels shown in corrosion casts of SEM by CLSM of uncorroded cast lymph nodes (Fig. 3).

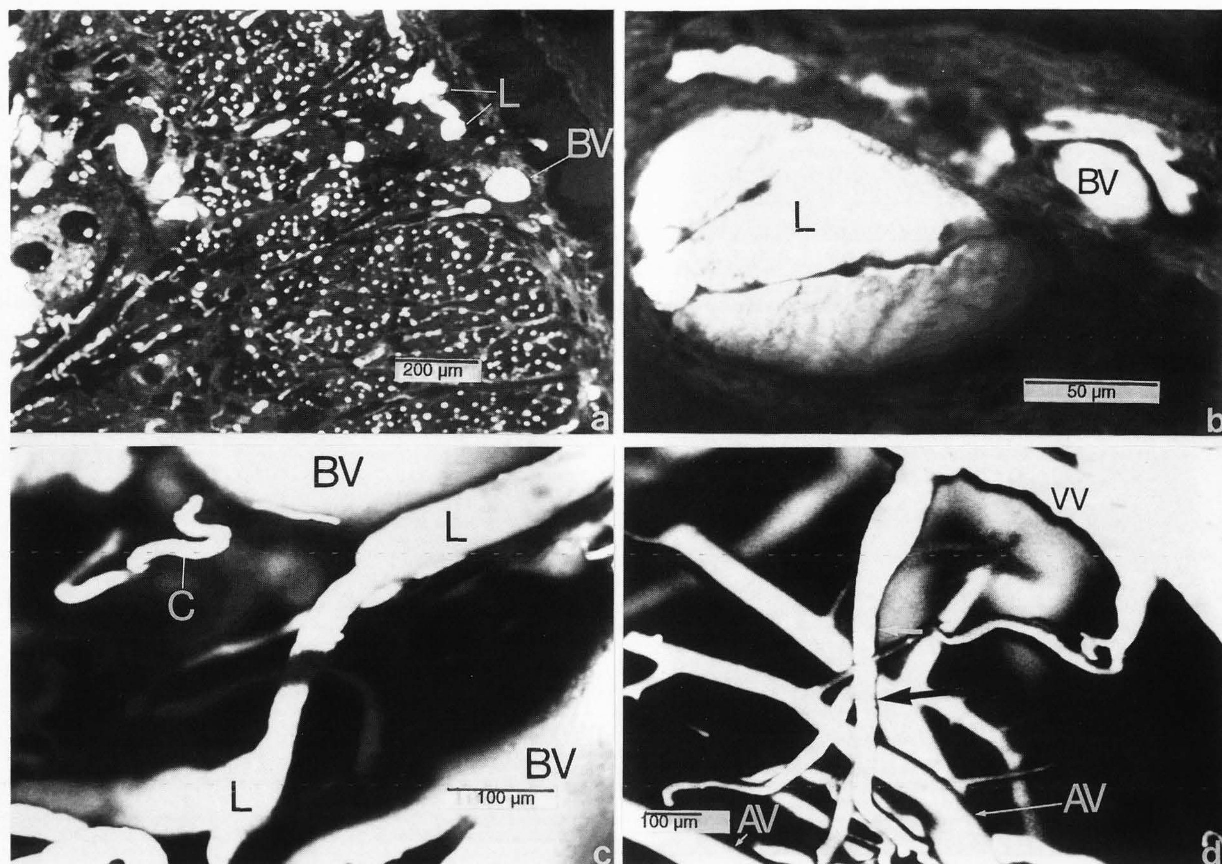

Figure 6. These confocal laser scanning micrographs present some examples in which the double filling technique of fluorescent resin was used. Mercox, stained with fluorescent yellow, was injected into the arterial system and Mercox rhodamine into the interstitial system. As a common result, in uncorroded tissue specimens, blood vessels and lymphatics can be well distinguished in the CLSM. (**a**) Cast tongue of the rat. Blood vessels appear in the original color micrograph as bright green-yellow fluorescent structures (BV), while lymphatics fluoresce reddishly (L). (**b**) Higher magnification micrograph of an uncorroded cast specimen. The cast of a lymphatic (L) contains a valve. BV corresponds to green colored casts of blood vessels. (**c**) In a corroded specimen, in the original color micrograph, the lymphatics (L) appears as red-colored cast structures together with the green-stained casts of blood capillaries (C) and larger blood vessels (BV). (**d**) Corroded specimen filled simultaneously from the venous and the arterial side with differently stained Mercox. In the original color micrograph, the yellow venous vessels (VV) can be well differentiated from the green colored arterial vessels (AV). Along a small capillary bridge (arrow), a gradual transition from one color zone to the other is recognizable.

CLSM of double-filled tissue

In one series of experiments, two fluorescent resins, one stained with rhodamine and the other with fluorescent yellow, were injected simultaneously but at different sites. If the injections are given into both the arterial system and the interstice of an organ, the blood vascular system is cast along with that of the interstitial spaces. Figure 4a shows a scanning electron micrograph of a corrosion cast in which this procedure was applied to the rat tongue. In such specimens, casts of blood vessels and interstitial spaces can be studied with respect to their individual morphological features as well as to their mutual topographic relationships. Additional information is obtained on cells and tissue structures surrounding the cast structures of both systems in uncorroded specimens by CLSM. An example is given in Figure 4b by the CLSM image of double-filled skin of the rat upper lip.

The double filling technique also seems to be useful to analyze the intricate spatial system of lymph nodes. Thus, in our experiments, this approach is used to distinguish the dense lymphoid tissue spaces from the nodal

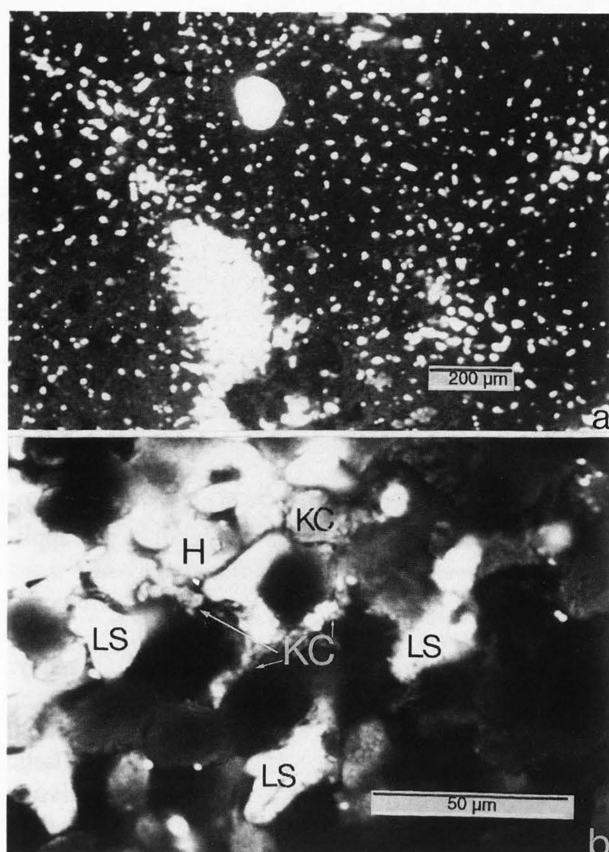

Figure 7. Confocal laser scanning micrograph of cast, uncorroded liver tissue. The liver was injected from the arterial side with Mercox rhodamine after prior treatment with interstitial injection of fluorescent microbeads. The specimen shows the liver sinuses partly filled with Mercox. A dot-like pattern produced by uptake of the fluorescent microbeads by the Kupffer cells is also noted. (**a**) At higher magnification, the cast liver sinusoids (LS) become visible together with single microbead-loaded Kupffer cells (KC) and hepatocytes (H). (**b**) Thus, in this specimen, a cellular reaction resembling phagocytosis is indicated by labelled cells together with vascular cast structures.

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sinuses and the elements of the nodal blood supply (Fig. 5). In addition, the structures filled by the resins could be examined in relation to the various nodal cell types surrounding them.

Further examples of the application of the double filling technique with reference to simultaneous filling of venous and arterial vessels in the blood circulation or lymphatics and blood vessels are demonstrated in Figure 6. In all these cases, CLSM of cast tissue specimens provides information on structural details, whereas SEM of corroded specimens cannot. By the dual detection system of the CLSM, a clear distinction between the two different fluorescent signals from rhodamine and fluorescent yellow with different excitation maxima is achieved. Thus, spaces and vascular tubes filled from different sites and belonging to separate systems can be easily examined and correlated with their tissue topography. They can be differentiated in casts, too.

Cast technique combined with cellular labelling

An approach to combine casting techniques with histophysiological investigations is presented now. In this series of experiments, the tissue selected for the subsequent casting procedure was first treated to demonstrate movements and phagocytosis of special cells (for further information on the histophysiological experiments carried out under different conditions, see [11]). In this paper, these phenomena are shown together with the vascular structures to which they are related (Figs. 3a, 7a and 7b). Fluorescent microbeads of 0.1-1.0 μm diameter were injected into the blood circulation for labelling of tissue macrophages or phagocytotic endothelial cells. Shortly after intravenous application, most of the injected fluorescent particles are captured by the cells of the reticular endothelial system (RES) and so are found as accumulated elements in liver, spleen, and bone marrow. The distribution of labelled macrophages in connective tissue or their migration along lymphatic pathways are also well-known phenomena occurring some days after interstitial application of the fluorescent microbeads. After the casting of these tissues, many of the labelled cells can be well recognized in the CLSM together with the cast vessels or tissue spaces with which they interacted (Fig. 7). Conventional staining of the cast tissue also allowed the identification of other non-phagocytotic cell types such as lymphocytes and erythrocytes. Further applications of this technique in studies of hemal lymph nodes in rats are reported elsewhere [13].

Discussion

In this paper, we have attempted to combine SEM of corrosion casts with light microscopy. The reason for such an approach was either to assess results so far exclusively derived from macerated specimens, or to get direct information on the casting procedure when the liquid resin fills a vascular system. The mode of casting microvascular structures of the blood circulation and the lymphatic system was first controlled by vital microscopy in our studies based on the rat tongue as model (unpublished). A similar approach was reported by Aharinejad *et al.* [7, 8], who used video microscopy in combination with corrosion casting (with SEM) applied to mouse exocrine pancreas.

In the present study, the technique of corrosion casting was applied along with laser scanning microscopy of fluorescent injected specimens. This approach has the following advantages:

(1). Tissue elements facing luminal casts before removal by maceration can be examined in cast uncorroded specimens by CLSM when fluorescent resin, such as Mercox, stained with rhodamine or fluorescent yellow is used. With the help of optical sectioning, even structural details surrounding the casts can be demonstrated. In addition to structures of the vascular wall region, those of the stromal tissue and parenchyma could be clearly demonstrated prior to the subsequent SEM examination of the corroded cast specimens.

(2). When resins stained with different fluorescent dyes are injected simultaneously, e.g., into an artery and a vein or into the interstice and the arterial system, arterial and venous vessels or blood and lymphatic vessels can be well distinguished in the CLSM. For that purpose, the dual detection system of that device is a very suitable tool.

(3). Both uncorroded and corroded specimens can be examined in the CLSM. Although, in the case of corroded specimens, information on the tissue components is no longer available, a good distinction between two differently stained cast systems can be achieved using the dual detection system of the CLSM.

(4). It could also be shown that CLSM allows us to obtain additional insight into kinetic processes visualized by labelled cells. This could be achieved in tissues where both techniques were used, i.e., that of casting and, prior to that procedure, labelling of macrophages by interstitial or intravenous injection of fluorescent microspheres into the living tissue.

The technique used makes it possible to gain hitherto inaccessible structural information on cast uncorroded tissues and organs in all SEM studies of vascular corrosion casts in which a precise morphological analysis of cast structures is needed. The technique also addresses the functional aspects of the vascular system in special cases. In this way, some misinterpretations of structures in SEM can be avoided and a better understanding of corroded cast specimens can be achieved.

Acknowledgements

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Discussion with Reviewers

A. Lametschwandtner: Did you find any interference of the fluorescent dyes with any known physico-chemical property of undiluted Mercox?

Authors: We did not find any interference with undiluted Mercox, which has to be contributed to the fluorescent dyes used. Thus, polymerization of Mercox and its replica quality have not been changed when fluorescent dyes were added.

A. Lametschwandtner: Do the fluorescent dyes bind to components of Mercox or do they stay in solution?

Authors: Generally, acridin-orange, rhodamine, and fluorescent yellow were added as powder substance to the liquid monomer and dissolved till saturation. It is unknown to us whether one of the dyes chemically binds to components of Mercox.

A. Lametschwandtner: For a later reexamination of uncorroded and corroded casts by CLSM, the fading of the fluorescent dyes is important. What was the fading time of the dyes you used?

Authors: The fading phenomenon of fluorescent cast structures is not very prominent in our specimens because the concentration of the dye in Mercox is high. Hence, specimens retain their fluorescence for as long as several months without noticeable reduction. This observation applied to both uncorroded and corroded cast samples as well.

A. Lametschwandtner: One of the advantages of CLSM is that by examining non-corroded cast specimens structural details as well as topographical relations can be studied, and that, in the light of these findings, SEM findings on corrosion casts can be discussed in a hitherto unachievable manner. To do so, however, one has to analyze the very same structures (e.g., a capillary and its surroundings) both by CLSM (before maceration) and SEM (after maceration). Please comment on the technical feasibility to study the very same details by both microscopies.

Authors: I agree that the examination of same specimens first observed as injected tissue specimens in the CLSM and then, after maceration, studied in the SEM as corrosion casts is a very desirable goal. The methodical approach described in this paper provides the possibility of comparing relevant structures existing around the cast medium with that of their replication occurring on cast surfaces.

G.J. Burton: Given the level of detail of the circulation which you have demonstrated here (e.g., the sphincter in Fig. 2), do you feel that corrosion casting provides any new information that cannot be gained by confocal microscopy?

Authors: The SEM provides images with a wide range of magnifications, and excellent resolution and contrast, qualities, which are never reached in CLSM. Thus, when applied to corrosion casts, SEM gives valuable information on the systemic nature as well as the structural details of special vascular segments like sphincters and other entities, although the findings are only based on indirect features like the imprint pattern of the endothelial cells and the dimensions of cast structures.

G.J. Burton: Since you are not corroding the fluorescent casts, is it necessary to use such a viscous medium as Mercox with all the associated problems of high perfusion pressures?

Authors: The viscosity of Mercox in comparison to other cast media is very low. Consequently, in our studies, Mercox has also proved very suitable for casting of specimen, which have not been subjected to the subsequent corrosion process.

G.J. Burton: Have you tried just using a fluorescent marker to endothelial cells following perfusion fixation to assess capillary density?

Authors: We have not tried such an approach as yet. From a fluorescent marker capable of yielding fluorescence effect, a special staining of the endothelial cells has to be expected. Otherwise, exact identification of capillary structures and, distinguishing them from other tissue cell components, seems very difficult. Cellular labelling of phagocytic cells, such as, lymphocytes or von Kupffer cells of the liver was performed by intravenous or interstitial injection of fluorescent microbeads (standard latex particles or liposomes) before corrosion casting was carried out.

P.A. Sims: How does the level of detail obtained with CLSM observation compare to standard corrosion casting; can the outlines of endothelial cell boarders be detected with the CLSM?
Authors: The representation of structural details such as endothelial nuclei or cytoplasm depends upon the fluorescent dye used. Some dyes provide nuclear staining, others stain the cytoplasmic zones including the cellular borders. But, these phenomena refer to the level of light microscopy and cannot be compared with the structural details appearing in SEM images taken at higher magnifications.

P.A. Sims: Since it has been reported that Mercox causes damage to endothelial cells, have you observed areas of vascular damage or change in the vascular wall in CLSM examined tissue?
Authors: We have not observed this phenomenon with the CLSM. To give a definite answer to your question, correlative SEM studies on cast tissue and uncorroded specimens will be required.

P.A. Sims: Is the glutaraldehyde fixation contributing to the fluorescein isothiocyanate (FITC) fluorescence, or have attempts been made to reduce the autofluorescence?
Authors: Glutaraldehyde fixation does not appear to interfere with the fluorescence of the dyes used in our study. With regard to the moderate autofluorescence of the fixed tissue, no attempts were made to reduce that phenomenon.

S. Aharinejad: How are plastic strips caused? We showed that Mercox penetrates into cells and mummifies them [5, 6]. Do you agree?
Authors: This problem has been discussed many times. Our interpretation of that peculiar phenomenon based on experimental studies has been reported elsewhere [14]. Mummification of certain cells by Mercox is one possibility to create the plastic strips. Other, subendothelial, plastic structures develop when Mercox penetrates the space between the basal lamina and the joining cells, myocytes and pericytes, and so fills up the space originally occupied by these cells.

S. Aharinejad: How do you know that you are dealing with endothelial cells or smooth muscle cells in CLSM? What are the criteria? Several structures are likely stained in Figure 2b as are those which are marked as endothelial smooth muscle cells.
Authors: Certain structural features, derived from commonly stained sections, such as the shape of the cellular nuclei and the position the cells, have criteria within the area of the vascular wall, which, in most cases, allow a clear distinction between endothelial and smooth muscle cells in our stained specimens.

S. Aharinejad: "Capillary sphincters" are not described and accepted as yet. We have shown that endothelial cells contract, and in this manner, contribute to blood flow regulation [8]. That evidence was provided by using intravital microscopy, a dynamic approach which convincingly demonstrates endothelial cells, myocytes, etc. What is the benefit of the static method you describe here versus intravital microscopy?
Authors: Certainly intravital microscopy seems to be the best way for the examination of functional processes occurring on the cytological level. CLSM can also be applied to that special field of microscopy, although the quality of the image is strongly influenced by disturbing events such as movements created by breathing and other sources. Thus, best results are obtained in supravital or fixed tissue stained with fluorochromes. On the other hand, the term "capillary sphincter", as used by us, refers to the very narrowed initial segment of special capillaries, thus, corresponding to the commonly used term "precapillary sphincter".