Short communication*

Approach to Combine Confocal Laser Scanning Microscopy with Casting Techniques of SEM

By

A. CASTENHOLZ

Department of Human Biology, University of Kassel, Heinrich-Plett-Str. 40, D-34109 Kassel, Germany

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In corrosion casts commonly examined in the SEM all cells and tissue components have been removed due the process of maceration. Thus, in such specimens, only the resin-filled lumina of vessels or tissue spaces appear as remaining structures. Although corrosion casts offer good insights into the systemic nature of the vasculature and interstitial spatial organization of a certain organ, no further information can be obtained on special morphological as well function data concerning these systems. Most investigators so are forced to make their description considering indirect criteria such as dimensions and topographic position of single vascular casts or imprints occurring on the cast surfaces. This, however, often leads to erroneous and speculations, which should be revised by additional procedures.

In pursuing that aim were made by us using resins stained with fluorescent dyes for the injection of vascular structure and tissue spaces. The so treated specimens proved very suitable for the examination on the level of both light and electron microscopy. Confocal laser microscopy was applied to resin-injected tissue specimens and those deprived from tissue by maceration. The results from these studies were compared with those of the SEM of the same specimens. Under these conditions important data could be collected about cells and tissue components surrounding vascular casts. Moreover a clear distinction was possible between casts of blood and lymphatic vessels and those of interstitial spaces. The innovative CLSM technique provided some additional benefits. Thus, by optical sectioning of injected tissue preparation, even the representation of individual cells begins in immediate contact to the luminal cast was possible. In this way the fine structural features

of precapillary sphincters could be analysed of CLSM of injected specimens referred to the possible of distinguishing two different fluorescent signals with the help of the two-detection system. Two differently stained resins (e.g. Mercox-fluorescent yellow and Mercox-rhodamin) could so be clearly recognized as separate cast structures in double filled specimens. Thus, a certain distinction between two compartments (e.g. vascular system and interstitial spaces or blood vessels) was achieved.

In our studies based on combined CLSM and SEM different organs such as tongue, skin, eyes and lymph nodes were examined in rats. So we were able to differentiate between vascular structures and tissue channels or between blood vessels and lymphatics, and also to clarify the cellular base existing around single luminal casts before maceration (Fig. 1a, b). With respect to that fact, the approach represented here provides additional information on corrosion casts in SEM and proves a useful tool in all cases, which need a precise interpretation of single cast elements. CLSM of fluorescent cells and tissue opens further ways to combine SEM casting method with the physiological experiment. Using the method of cellular labelling with fluorescent dyes, we were also able to get insights into kinetics of lymph node tissue, liver, spleen and other organs before these organs were subjected to special procedures such as casting and corrosion. Experiments like these may also contribute to a better understanding of the morphological as well as functional nature of case specimens represented in the SEM.

* Further remarks and data concerning the context of that contribution will published in SEM 1994. The author wants to dedicate both papers to Prof. Yoshikuni Ohta in appreciation of his pioneering work on dental and oral anatomy.

References

- 1) Castenholz, A. (1994): Control of corrosion casts by confocal laser scanning microscopy of tissue specimens. *Scanning Microsc.* 1994 (in press).
- 2) Castenholz, A. (1994): Confocal laser scanning microscopy applied to experimental lymphology. *Proc. SCANNING 94*, vol. 16/IV:35–36, FAMS, Inc. Mahwah, N.J., USA.

Explanation of Figures

Plate I

CLSM images of tissue specimens injected with a fluorescent resin (Mercox-acridin-orange)

Fig. 1a. The capillary system of the papillary layer of the rat tongue exhibits bright fluorescence and is represented along with other tissue elements.

Fig. 1b. In this high power micrograph with an optical section through a ciliary process of the rat eye even single cellular elements of the epithelial layer around the cast of the capillary are recognizable.