Staining Capillaries in the Myocardium for Synchrotron Radiation-based Micro Computed Tomography

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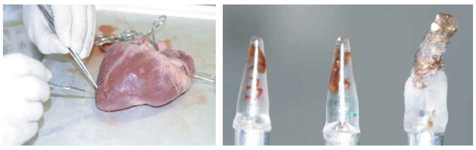
INTRODUCTION

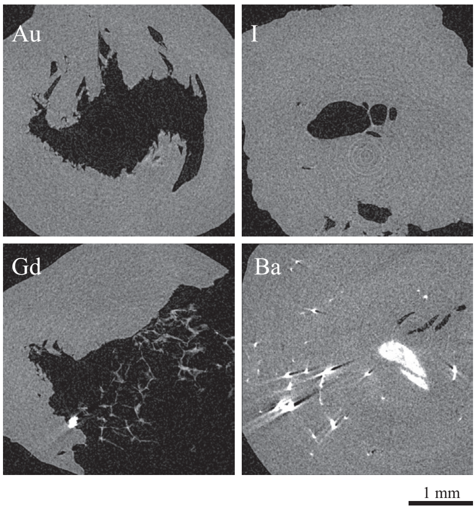
Blood vessels formed after medical interventions such as radiofrequency treatment have to be visualized down to the capillary level to validate the procedure. Synchrotron radiation-based micro computed tomography (SRµCT) provides the necessary micrometer resolution, but since the vessels and the surrounding tissue consist nearly exclusively of water the absorption contrast is too weak to segment the vessel tree. Therefore, suitable staining protocols have to be developed. Our study proved that lyophilic salts with the mean particle diameter of 1.5 µm such as $BaSO_4$ are well suited to stain even the smallest vessels, namely the capillaries. The combination of these salts with the embedding kit JB-4 (Polysciences Inc.) allows tissue fixation and long-term storage without time-consuming water replacement.

TISSUE PREPARATION



The injection of the mixture of JB-4 polymer and stain (Au, Ba, Ca, Gd, I, Os, Sr) into the arteries can be followed by eyes because the color of the tissue slightly changes from red to white-yellow. Hence, the region of interest can be easily selected and cut out using the scalpel. During the injection the viscosity of the mixture increases. The set of embedded samples demonstrates that the use of the Eppendorf containers to fix the entire tissue sample is an appropriate preparation method.

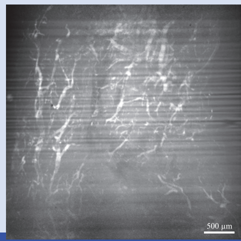
STAIN SELECTION

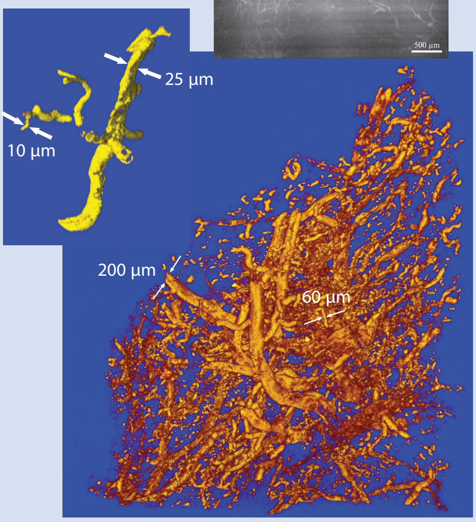


In order to get the overview of the differently stained samples and the suitability of the staining materials for the blood vessel visualization by high-resolution micro computed tomography, conventional micro computed tomography (µCT 40, Scanco, Switzerland) using 40 kV for the X-ray generation was performed. Here, the support of the team of R. Müller (ETH Zürich) is gratefully acknowledged. The µCT-slices show that the sulfates are best suited to make visible the smaller blood vessels (some 10 µm in diameter).

2D AND 3D VISUALIZATION

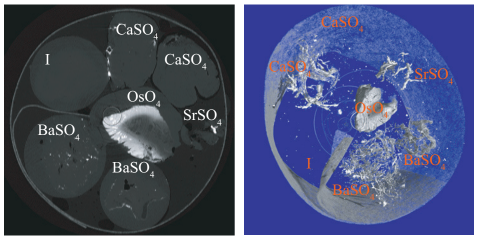
Another possibility to identify promising staining materials and procedures is radiography. The projection of the $BaSO_4$-stained sample very clearly shows the complex network of blood vessels in the myocardium. The image was acquired at the materials science beamline 4S (SLS, Villigen, Switzerland) using the photon energy of 10 keV and the illumination time of 2.6 s. Note that the instability of the synchrotron X-ray beam resulted in intensity modulations, although the image has been background corrected.





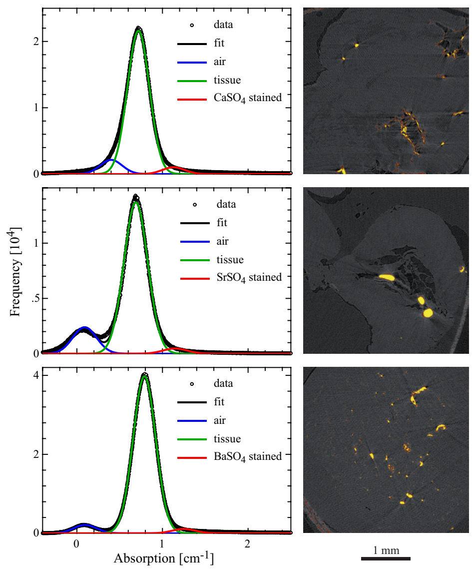
The SRµCT-data of the $BaSO_4$-stained sample enable the intensity-based segmentation of the vessel tree in the myocardium. Vessels with diameters between tenths of millimeter and 10 µm are identified. The thinnest parts of the yellow-colored vessels shown as the inset represent capillaries with diameters of 2 voxel lengths (2x5 µm). The 3D representations show that the vessels are often disconnected. Therefore, we conclude that the stain is not homogeneously distributed especially within the thinner vessels.

SRµCT



Seven specimens were combined to realize a reasonable total absorption value of the sample at the lowest possible photon energy at the beamline W2 (HASYLAB at DESY, Hamburg, Germany) of 20 keV. The simulataneous measurement allows the direct comparison of the specimens.

HISTOGRAM ANALYSIS



To determine the threshold for the vessel segmentation, the histograms of the absorption values within the region of interest were analyzed. From previous studies we knew that each component exhibits the Gaussian distribution. If the components are enough separated, the crossing point is an appropriate choice for the threshold. The histograms indicate that the 3 sulfates are suited for the staining of the blood vessels in the myocardium.

CONCLUSION AND ACKNOWLEDGEMENT

The blood vessels of freshly explanted myocardium of pigs can be stained by sulfates of Ca, Sr, and Ba suspensed in the embedding kit JB-4. Hence, a preparation protocol is developed for long-term storage of locally stained biological tissues. The 3D data-sets of SRµCT allow the rather simple intensity-based segmentation of the blood vessels down to the capillaries. Because the vessels in the 3D representations appear sometimes disconnected, the procedure has to be further improved, e.g. by tailoring the suspension with respect to particle size and density as well as the injection of the mixture into the arteries.

The authors thanks U. Dietz (German Clinics for Diagnistics, Wiesbaden, Germany) for initiating the project. The authors thank the team at the veterinary faculty of the Zurich University, especially H. Augsburger, E. Bürgi, and U. Müller for providing the fresh porcine hearts. The support of M. Stampanoni (SLS Villigen, Switzerland) at the materials science beamline is gratefully acknowledged. The project was partially funded by Medtronic, Maastricht, Netherlands, by the Swiss National Science Foundation (Grant No. 2153-057127.99), by DESY, Hamburg, Germany (proposal I-02-068) and by SLS, Villigen, Switzerland (proposal 20030152).