Vessel staining in tumours by Angiofil®

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INTRODUCTION: In cancer research imaging the blood vessels of tumours plays an important role to investigate the angiogenesis. Micro-computed tomography (µCT) provides the spatial resolution to image the smallest vessels, but not the necessary contrast. Therefore, one has to apply appropriate embedding methods[1] or contrast agents such as barium sulfate.[2] As the particle sedimentation results in "non-connected" vessels,[2] alternative contrast agents are desired. The iodine-based contrast agent Angiofil®[2] belongs to the promising species and is used in the present study.

METHODS: C51 tumour cells were injected in nude mice in strict adherence to the Swiss law for animal protection. Using Magnetic Resonance Imaging (MRI) the tumour was investigated during growth. 200 µl heparin were injected before perfusion. Subsequently, the tumour was filled with the prepared Angiofil® solution via the left ventricle of the heart using the peristaltic pump. Finally, the tumours were extracted, fixed in 4% para-formaldehyde and transferred to the Eppendorf tubes for the imaging. The stained vessels were visualized by means of synchrotron radiation-based micro computed tomography (SRµCT) at the beamline TOMCAT (SLS at PSI, Switzerland) in absorption contrast mode.[3] The photon energy was set to 18 keV. A series of 1501 projections with a pixel size of 3.8 µm was recorded.

RESULTS: Fig. 1 shows the 3D representation of the vascular structure within the Angiofil®-perfused tumour. One clearly recognizes that the stain is concentrated in some vessels. Bifurcations are hardly visible. The vessel tree is only inhomogeneously stained. Nevertheless, it is seen that the centre of the tumour is almost free of connected vessels. The stained vessels have diameters between 8 and 400 µm .

DISCUSSION & CONCLUSIONS: Angiofil can be used to stain vessels including the bigger capillaries in tumour tissue. The procedure, however, has to be improved to obtain the homogeneous distribution of the stain within the vessel tree and the penetration of the smallest capillaries. The analysis of the vessel diameter is required for modelling of the fluid dynamics as well as for the calibration of the in vivo MRI. Therefore, the search for appropriate staining materials has to be continued, in order to quantify the tumour in reproducible way. The missing stained vessels in the tumour centre are associated with the necrotic part of the tumour. Alternatively, one could apply phase contrast imaging avoiding any staining procedure.[4]

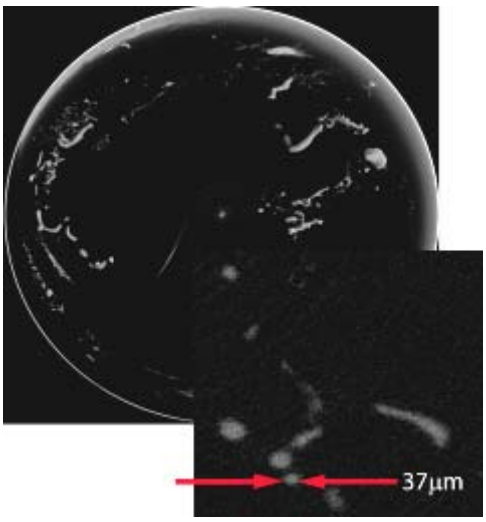

Fig. 1:The 3D representation, generated by VGStudio MAX shows the central part of the tumour (d = 7.8 mm), while only at periphery vessel structures appear. The fraction of the virtual slice exhibits different vessels, one 37 µm thick is highlighted.

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