A Multilevel Framework to Study The Altering Hepatic Circulation in Cirrhotic Rats.

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ABSTRACT

AIM: Liver cirrhosis is a chronic disease of the liver, severely affecting the hepatic architecture and liver functions. To date, little is known about the hemodynamic consequences caused by cirrhosis, especially at the microscopic level. In order to analyze the altering morphology and perform computational flow simulations, detailed 3D reconstructions of the hepatic microcirculation are essential. In this work, we present two techniques which enable acquiring accurate 3D geometrical data of the rat liver (micro)circulation, namely vascular corrosion casting supplemented with µCT-imaging, and immunohistochemistry (IHC) combined with confocal laser microscopy.

MATERIAL & METHODS: The vascular corrosion casting technique entails injecting the casting resin (PU4ii; VasQtec) in the hepatic artery (HA) and portal vein (PV). Lipiodol (Guerbet) is added to the arterial mixture as a contrast agent to ensure a clear distinction between both vascular trees while µCT-imaging the vascular casts (Fig 1a). The resulting datasets are subsequently processed using segmentation software to create anatomical 3D reconstructions of the hepatic macro- and microcirculation.

The IHC protocol includes whole animal perfusion fixations prior to immunostaining. The staining is performed on 300 µm thick liver slices with the antibody RECA (Rat Endothelial Cell Antigen; Serotec) and the fluorescent dye Cy3. To increase the liver slice's transparency and visualization depth, the clearing protocol CUBIC is applied, which minimizes light scattering while preserving fluorescent signals. Next, image stacks are recorded with a confocal microscope at submicron resolutions. Software is developed to automatically process and segment these datasets, enabling the visualization of the liver network in 3D (Fig 1b).

RESULTS and CONCLUSIONS: The aforementioned complementary techniques provide a methodological framework to study the changing morphology of the hepatic (micro)circulation in cirrhotic rats, which may lead to new insights in the pathophysiology of liver cirrhosis. In addition, the reconstructed 3D vascular network will be used for CFD simulations to assess the hemodynamic changes due to cirrhosis.

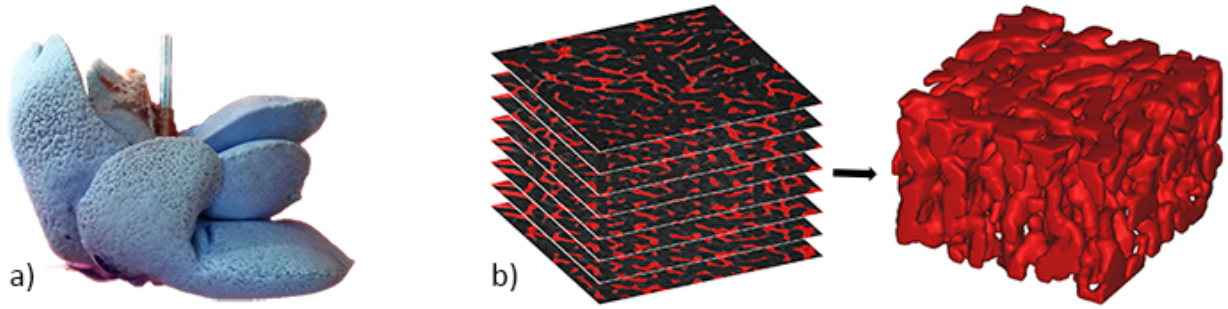

Fig 1: a) Vascular cast of a rat liver. b) Processed and segmented IHC datastack.