Multi-level modelling of angioarchitectural alterations during liver cirrhogenesis in rats

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The hepatic circulation plays a crucial role in liver (dys)function and diseases such as liver cirrhosis. The latter severely affects the hepatic vasculature as normal liver tissue gets replaced by fibrosis, regenerative nodules, and vascularized fibrotic septa. To date, morphological vascular alterations caused by cirrhogenesis are not fully understood, especially at the microlevel. It is thus essential to further explore the hepatic angioarchitecture and hemodynamics going from healthy to fully cirrhotic conditions. To this end, we imaged and modelled the vasculature of the rat liver during cirrhogenesis across different length scales.

The previously developed thioacetamide protocol [1] was used to chemically induce cirrhosis in rats (n=36). Thioacetamide intoxication typically results in centrilobular necrosis and homogenous macronodular cirrhosis after approximately 18 weeks. Rats were randomly assigned to 4 groups (n=9) and sacrificed after 0, 6, 12, 18 weeks, respectively. Within each group, 5 rats were used for vascular corrosion casting combined with micro-CT scanning and 4 rats for immunohistochemistry combined with deep tissue microscopy.

During vascular corrosion casting, resin (PU4ii) was injected in the hepatic artery and portal vein (Fig. 1 a,d). Lipiodol was added to the arterial mixture as a contrast agent to enable a clear distinction between arteries and veins after high resolution micro-CT-scanning. The resulting datasets were processed to create detailed anatomical 3D reconstructions of the hepatic (macro)vasculature (Fig. 1 b,e). Dedicated software [3,4] which builds on TiQuant [5] allowed for automated processing of these datasets. Analysis of the vascular branching topology was based on a top-down diameter-defined ordering method and morphological parameters (radius, tortuosity, length, etc.) were quantified.

The immunohistochemistry protocol started with whole rat perfusion fixations prior to immunostaining, which was performed on 350 µm thick liver slices with a generic endothelial marker antibody (RECA). To increase the liver slices' visualization depth, the clearing protocol CUBIC [2] was adjusted and applied. Subsequently, image stacks were recorded with a confocal microscope. The same dedicated software package as mentioned above enabled segmenting and analyzing the liver microcirculation network (Fig. 1 c,f) [3,4].

At the macrolevel, we noticed that regenerative nodules severely compressed pliant venous vessels (especially hepatic veins) from 12 weeks thioacetamide intoxication onwards, showing

collapsed vessel segments severely reducing perfusion capabilities. HA vessels became more tortuous during cirrhogenesis with the appearance of sharp bends, which were absent in the control rats (0 weeks) [4].

At the microlevel (Fig. 1 c,f), cirrhogenic alterations clearly affect the hepatic perfusion as the porosity (i.e. the volume percentage of sinusoids) decreases from 20.8 ± 2.3% (0 weeks) to 11.4 ± 3.1 % (18 weeks). Furthermore, the mean sinusoidal radius was significantly lower ($p<0.05$) in cirrhotic (3.9 ± 0.4 µm) compared to normal liver tissue (4.4 ± 0.3 µm). Our data also suggest a zone-specific sinusoidal degeneration with sinusoids located near the liver capsule being more affected than those in the middle of a liver lobe [4].

The developed methodological framework enabled studying the morphological alterations of the hepatic circulation during cirrhogenesis across different length scales. The reconstructed 3D vascular networks may be used to develop multi-level numerical modeling approaches (e.g. lumped parameter models, computational fluid dynamics) to study cirrhogenic hemodynamics. This may lead to novel insights in cirrhotic pathophysiology.

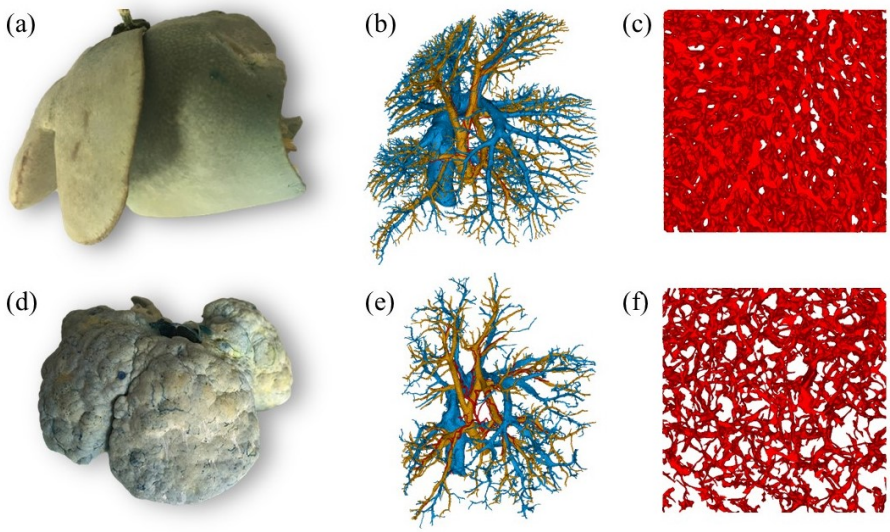

Figure 1: Illustration of the experimental methods and data analysis for a normal (0 weeks, panels a-c) and a cirrhotic rat liver (18 weeks, panels d-f): (a,d) vascular corrosion cast of a whole liver, (b,e) 3D reconstruction of a cast showing the hepatic arterial (red), portal venous (orange) and hepatic venous (blue) vascular trees, (c,f) 3D reconstruction of sinusoids based on a deep tissue microscopy dataset.

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