A multilevel framework to study the adaptive vascular morphology during liver cirrhogenesis

***Authors (max 5.)**: Geert Peeters[1], Charlotte Debbaut[1], Pascal Verdonck[1] , Wim Laleman[2], Patrick Segers[1]*

[1] IBiTech – bioMMeda, Department of Electronics and Information Systems, Ghent University, Belgium
[2] Hepatology, University Hospitals Leuven, & Department of Liver and Biliopancreatic disorders, KU Leuven, Belgium.

Abstract:

Liver cirrhosis forms the final stage of any chronic active liver disease ultimately leading to hepatocellular failure and aggravated pressure in the portal venous system. These latter form the backbone of all potential and often lethal complications. To date, little is known about the interplay between the progression of fibrosis to cirrhosis and its vascular consequences, especially at the cellular level. To gain more insight in the impact of the angioarchitectural disarrangement on the hemodynamics, accurate 3D reconstructions of the hepatic (micro)circulation are pivotal. In this study, we have optimized two complementary techniques to acquire accurate 3D geometrical data of the rat liver circulation, covering the entire length scale of the hepatic vasculature, and applied it to an established rat model of cirrhosis [1].

a) Vascular corrosion casting (VCC) entails injecting a casting resin (PU4ii) in the rat hepatic artery and portal vein. Lipiodol is added to the arterial mixture as a contrast agent to ensure a clear distinction between both vascular trees on micro-CT-scans. The resulting datasets are processed with Mimics (Materialise) and enable reconstructing detailed 3D geometries of the hepatic macro- and microcirculation (see Figure).
b) The immunohistochemistry (IHC) protocol includes staining 350 µm thick liver slices with a generic endothelial marker antibody (*RECA)*. To increase the liver slices' transparency and microscopic penetration depth, a modified version of the clearing protocol CUBIC is applied. Image stacks are subsequently recorded with a confocal microscope, and automatically segmented to visualize and analyze the microcirculation using in-house developed software.

We were able to gather and compare morphological parameters (radius, tortuosity, length, etc.) during cirrhogenesis. Our initial results suggest that - even in the advanced fibrotic stages - microcirculatory alterations manifest as the porosity (i.e. the mean number of liver-specific capillaries (sinusoids) per unit of volume) reduces from 20.35 ± 1.20% (normal) to 16 ± 1.73 % (early stage fibrosis), while the mean radius remains similar for early stage fibrosis (4.88 ± 0.13 µm) compared to normal liver tissue (4.79 ± 0.22 µm).
This multilevel framework allows quantifying the impact of cirrhosis on the hepatic angioarchitecture and may lead to novel insights in the cirrhotic pathophysiology. Later on, the detailed 3D geometries will serve as input for numerical simulations to study the hemodynamics of cirrhosis.

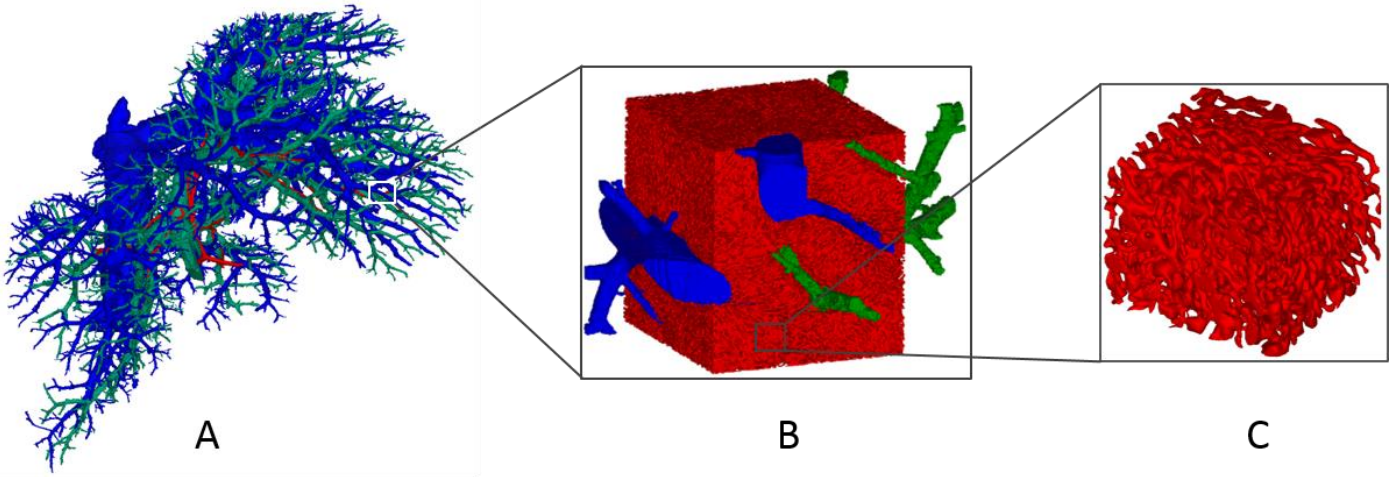

Figure caption: Multilevel 3D reconstruction of the hepatic vasculature in a rat. A. Macrocirculation including the hepatic artery (red), the portal vein (green) and the hepatic veins (blue). B. Microcirculation with afferent (green) and efferent (blue) vessels. C. 3D reconstruction of the liver-specific widened capillaries (sinusoids).

References:

1. Laleman, W, et al. "A stable model of cirrhotic portal hypertension in the rat: thioacetamide revisited." *European journal of clinical investigation* 36.4 (2006): 242-249.