

3D IMAGING AND MORPHOMETRY OF THE CORONARY MICROCIRCULATION IN SPONTANEOUSLY HYPERTENSIVE RATS AND NORMOTENSIVE CONTROLS

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Several causes, both genetic and environmental, can lead to development and progression of arterial hypertension. Up to date, our knowledge on vasculature remodeling associated with arterial hypertension is based on non-integrated information commonly obtained with low spatial resolution methods. Here, we investigate the coronary microcirculation by developing a protocol to perform high-resolution 3D structural reconstruction to define the role of its anatomical remodeling in the context of hypertension.

Retrograde perfusion of rodent hearts was optimized to introduce a fluorescent gel in the coronary circulation. Subsequently, the fluorescent gel solidified producing a 3D cast of the entire cardiac vasculature. An optimized CLARITY-based tissue clearing protocol was employed to render the heart transparent thus allowing a 3D mesoscopic reconstruction at the capillary level using two-photon fluorescence microscopy.

We applied the protocol on Spontaneously Hypertensive Rats (SHR), an established animal model of hypertensive cardiomyopathy characterized by significant remodeling of the coronary vascular network. We quantified the rate of vascular remodeling over time by analyzing vascular morphometries of normotensive controls and SHR rats at different ages. The 3D microcirculation reconstructions were analyzed to quantify geometrical properties of the capillary networks in term of sections, linear density, fractality, and angular dispersion. We found significant remodeling of coronary microcirculation in SHR at all ages, including at four weeks old where linear density and angular dispersion significantly increased while no blood pressure alteration was observed.

The methodology presented here demonstrated the possibility of reconstructing the coronary microcirculation in 3D with high resolution, enabling the study of the cardiac anatomic signatures in both physiological and pathological conditions, thus offering a reliable method for integrated quantitative analyses.