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Structural imaging and characterization of the arterial wall and its perivascular adipose tissue

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Introduction

Blood vessels possess a lumen, where blood circulates, and a wall made of three layers: intima, media and adventitia. These tunica comprise cells surrounded by a dedicated extracellular matrix (ECM) providing scaffolding and structural support for them. Elastin is the ECM protein imparting elasticity to the arterial wall. Its abundance in the media layer. Besides, the arteries are surrounded by perivascular adipose tissue (PVAT) also providing mechanical support to the vascular wall and controlling vascular physiology (1). PVAT is essential to maintain vascular hemostasis both mechanically and by endocrine/paracrine ways.

Aging is an important risk factor for cardiovascular diseases development. In pathological and physiological conditions, aging is also associated with both ECM (2) and PVAT loss of functions, due to remodeling and expansion respectively.

Objectives

Vascular pathologies are accompanied by structural and functional modifications which affect the arterial wall and PVAT. The aim of the present study is to determine how ECM remodeling occurs during healthy and pathological aging by detecting, measuring and quantifying faint structural modifications in the arterial wall (3), and surrounding PVAT

Materials and methods

Samples



- 6-months old male C57BL6 mice (control, *db/db*).
- Aorta were harvested as follows: after euthanasia, heparin was injected. Heart and aorta were then flushed with PBS using a syringe driver to remove residual blood. The aorta was prefixed for 5 min. After that time, low melting agarose was injected.
- Fixation of heart, aorta and PVAT for 24–48 h.
- Paraffin embedding.

Image acquisition and reconstruction

a) Imaging segments of aorta using synchrotron X-ray micro-computed tomography without contrast agent in different anatomical regions (thoracic vs abdominal aorta).



SOLEIL synchrotron



Sample paraffin embedding

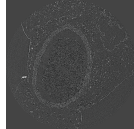


Image acquisition

b) Image reconstruction (0.65 μm, 2048 x 2048 pixels)



control



db/db

Results

Image processing

We distinguish elastic structures in the arterial wall at a resolution and size never achieved before (0.65 μm) and we visualize PVAT ECM.

Arterial wall and PVAT segmentation

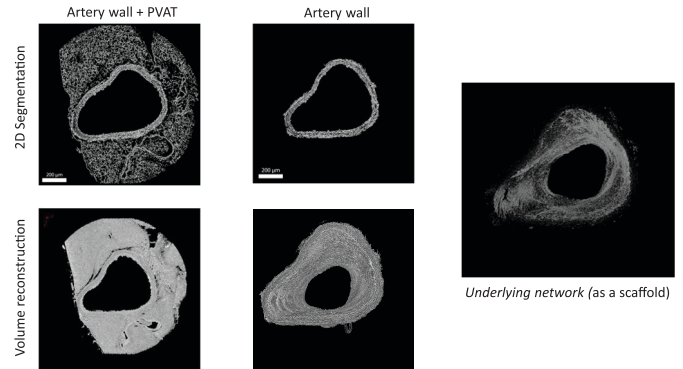
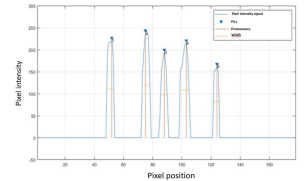
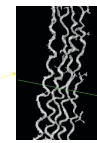
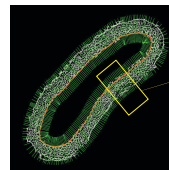


Image analysis

Methods for assessing parameters

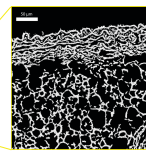
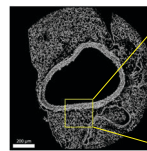
- Arterial wall : intensity profiles (more than 80k measurements / sample)



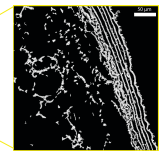
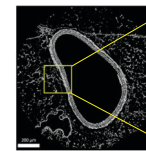
- PVAT : density

$$\text{Ratio} = \frac{\text{network area}}{\text{total area}}$$

Control vs diabetic



control



db/db

➤ Our algorithm can extract informations from the elastic structures:

- No significant differences are observed in elastic lamellae thickness between diabetic and control mice.
- In diabetics mice, lamellae are smooth and stretched while they are wavy in control mice.
- PVAT ECM network is denser and better structured in control mice.
- PVAT ECM density: 21.8 ± 2.4 % in controls 9.3 ± 1.1 % in diabetic mice.
- Expansion and degradation in diabetic mice.

Discussion and conclusion

Tissue characterization is a fundamental tool for studying and diagnosing pathologies and lesions associated to the vascular tree. Our automatic algorithm is robust and permits the extraction of informations from arterial structures. We can image a segment of aorta in 3D in a reproducible manner without cutting the sample.

Our results show that important structural differences exist between vascular and PVAT ECM in diabetic vs control mice. These modifications support the view that the diabetic pathology precipitates vascular aging.

Bibliography

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