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

Aicha Ben Zemzem<sup>1</sup>, L. Vanalderwiert<sup>1</sup>, X. Liang<sup>1,2</sup>, S. Blaise<sup>1</sup>, T.Weitkamp<sup>3</sup>, M.Dauchez<sup>1</sup>, N. Passat<sup>2</sup>, L.Debelle<sup>1</sup> and S.Almagro<sup>1</sup>

- UMR CNRS 7369 Matrice Extracellulaire et Dynamique Cellulaire, SFR CAP Santé, Université de Reims Champagne Ardenne, UFR Sciences Exactes et Naturelles, moulin de la housse, BP
- 1039, 51687 Reims cedex 2, France,
  CRSTIC EA 3804 Université de Reims Champagne Ardenne, 51687 Reims cedex 2, France.
  Synchrotron SOLEII, 91192 Gif-sur-Yvette, France

#### Introduction

Blood vessels possess a lumen, where blood circulates, and a wall made ofthr ee layers: intima, media and adventitia. These tunica comprise cells surrounded by a dedicated extracellular matrix (ECM) providing scaffolding and structural supportfor them. Elastin is the ECM protein imparting elasticity to the arterial wall. Itis abundan tin the media layer. Besides, the arteries are surrounded by perivascular adipose tissue (PVAT) also providing mechanical supportto the vascular wall and controlling vascul ar 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 offunctions, due to remodeling and expansion respectively.

# Objectives

 $oldsymbol{V}$ ascular pathologies are accompanied by structural and functional modifications which affects the arterial wall and PVAT. The aim ofthe present study is to determ ine how ECM remodeling occurs during healthy and pathological aging by detecting, measuring and quantifying faint structural modifications in the arterial wall. 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 thattime, low melting agarose was injected.
- Fixation ofheart, 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 agentin differen t anatomical regions (thoracic vs abdominal aorta).





Sample paraffin embedding



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



control



dh/dh

#### Results

# Image processing

 $\mathbf{W}$ e distinguish elastic structures in the arterial wall at a resolution and size never achieved before (0.65  $\mu\text{m})$  and we visualize PVAT ECM.

#### ☐ Arterial wall and PVAT segmentation







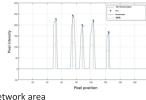
Image analysis

#### ■ Methods for assessing parameters

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







PVAT : density

Ratio =

network area total area

# □ Control vs diabetic









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 FCM network is denser and better structured in control mice.
- ECM **PVAT** density: 21.8 2.4 % controls  $9.3 \pm 1.1$  % 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 ofinformations from arterial structures. We can image a segment of aorta in 3D in a reproducible manner without cutting the sample. Our results show thatimportant structural differences exist between vas cular 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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