OPTIMIZATION OF MICROCT AND CONTRAST-ENHANCED MICROCT FOR CARDIOVASCULAR APPLICATIONS

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Introduction

Atherosclerosis is a leading cause of death. To better understand and treat this disease, it is important to evaluate the changes in blood vessel wall microstructure during disease development and to link these structural changes to changes in mechanical properties. Microfocus X-ray computed tomography (microCT) is a powerful technique for assessing, in a non-invasive way, the 3D microstructure of complex materials. To achieve sufficient X-ray attenuation in soft tissues, contrast-enhancing staining agents (CESAs) containing high atomic number molecules are combined with microCT. This technique is referred to as contrast-enhanced microCT (CE-CT). Monolacunary and Hafnium-substituted Wells-Dawson polyoxometalate (Mono-WD and Hf-WD POM respectively) have shown their potential for non-destructive staining of kidney and soft skeletal tissues [1]. Additionally, we have shown with planar biaxial testing that these POMs have a negligible effect on the mechanical properties of the tissue. In this study, both CESAs were evaluated and compared for their staining potential of the porcine aorta. Other types of blood vessels were imaged to show the ability of CE-CT to highlight the vessel wall microstructure, including the different wall layers and their content in elastin and collagen. The novelty of this study lies in the high-detailed comparison of the microstructure of different types of blood vessels (healthy and diseased) in a non-invasive way.

Materials, methods and results

Samples of 1x1cm of porcine aorta were fixed. A control scan and scans at different time points after staining with Mono-WD POM or Hf-WD POM were performed. The grayscale value was measured at different locations inside the tissue (x). This data was then fit to an exponential decay equation [2]:

$$I = I_0 + (I_{max} - I_0)\left(1 - e^{-kt}\right) \qquad (1)$$

where I_0 is the grayvalue of the control sample, I_{max} is the grayvalue after complete staining, and t is the staining time. From this experiment, we concluded that Hf-WD POM stains with much higher intensity and allows a better contrast between the different vessel wall layers while Mono-WD POM stains less bright and more homogeneously but diffuses much faster than Hf-WD POM (Fig. 1).

The optimized staining parameters were then applied to other types of porcine blood vessels (elastic artery, muscular artery, and vein) and allowed to observe the differences in the 3D microstructure of the vessel wall in these tissues. We also showed important species-related (porcine, rat, human) differences in the vessel wall microstructure of the aorta, which can be related to changes in functional behavior. From the data mentioned above, 3D morphometrical parameters can be obtained without altering the native structure of the tissue. Fig. 2 shows a CE-CT based region of interest of a segmented rat aorta from which the elastin volume fraction and elastic fiber waviness and separation can be determined. Finally, healthy and atherosclerotic human femoral arteries were compared. Plaques and onsets of calcifications can be identified and parameters such as calcification volume fraction can be quantified.

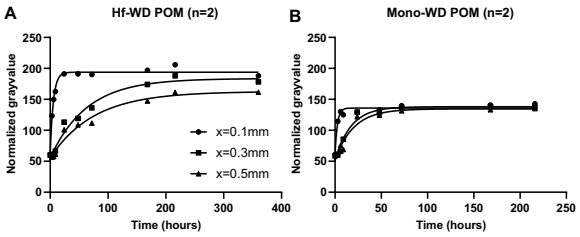

Fig. 1: Normalized grayvalue in function of time for different locations inside the tissue and fitted with the curve from equation 1 for (A) Hf-WD PM and (B) Mono-WD POM.

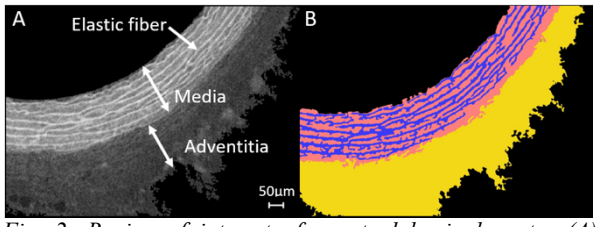

Fig. 2: Region of interest of a rat abdominal aorta: (A) original grayscale slice and (B) segmentation of the different substructures of the aortic wall. Yellow = adventitia, blue = elastic fibers, pink = smooth muscle cells and collagen matrix

Conclusion

CE-CT has shown its potential for high-resolution analysis of the vessel wall layers and their composition. In order to better understand the blood vessel mechanical properties, CE-CT can be combined with *in situ* mechanical testing (4D CE-CT). In the future, this data can then be used as input to obtain more accurate 3D computational models which help to understand the effect of changes in microstructure on functional behavior.

References

[1] de Bournonville et al, Acta Biomater., 105:253-62, 2020
[2] Balint et al, PloS One, 11(4):e0153552, 2016