Screening staining agents for contrast-enhanced microCT of vascular tissues: a microstructural and mechanical analysis as input for improved design of tissue-engineered vascular grafts

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Currently available synthetic vascular grafts have contributed to improved outcomes in cardiovascular surgery. However, they still lack in mimicking the complex mechanical behavior of vascular tissues, such as the non-linearity, the anisotropy, and the viscoelasticity. These complex mechanical properties are due to a highly heterogeneous tissue microstructure, which is composed of collagen fibers and elastic lamellae that unfold and stretch at different strains. Tissue engineered (TE) vascular grafts could significantly improve the treatment outcomes. However, for an accurate design of these grafts, there is a need for better understanding the relationship between the microstructure and the mechanical properties of vascular tissues. For this reason, 4D-contrast-enhanced microfocus X-ray computed tomography (CECT) imaging can be used. It combines in situ mechanical loading with 3D microstructural visualization of arterial tissue. Since absorption-based CECT requires the use of contrast-enhancing staining agents (CESAs), we investigated six contrast-enhancing staining agents (CESAs) for their suitability for 4D-CECT of arterial tissue. Indeed, a suitable CESA should: i) provide good contrast enhancement, ii) allow accurate visualization and segmentation of the tissue constituents present in vascular tissues and iii) should not alter the mechanical properties of the tissue.

Our study focused on three criteria for microstructural imaging: CESA penetration speed, contrast enhancement, and ease of segmentation of elastic lamellae. Additionally, we assessed the effect of CESAs on the mechanical properties of, considering volume change and stiffness. Phosphotungstic acid and Lugol iodine, commonly used CESAs with excellent contrast enhancement, were unsuitable for 4D-CECT due to tissue shrinkage and stiffening effects. Among the remaining four CESAs, all known as polyoxometalates (POMs), Hafnium-substituted 1:2 Wells-Dawson POM (Hf) showed the most favorable behavior, not affecting the mechanical properties. It emerged as the preferred CESA for 4D-CECT of arterial tissue, offering superior contrast enhancement and ease of elastic lamellae segmentation. Furthermore, higher resolution imaging was performed on arterial tissue with Hf and microstructural analysis was done on the elastic lamellae thickness and separation. These findings provide valuable insights for (4D-)CECT imaging of arterial tissue.

In future work, we will apply 4D-CECT to native tissues to investigate the link between microstructure and mechanical properties. This approach will enable us to perform comparative analyses between TE vascular grafts and native vascular grafts to validate the design and establish a quality control protocol for these of TE grafts prior to their implantation in patients.

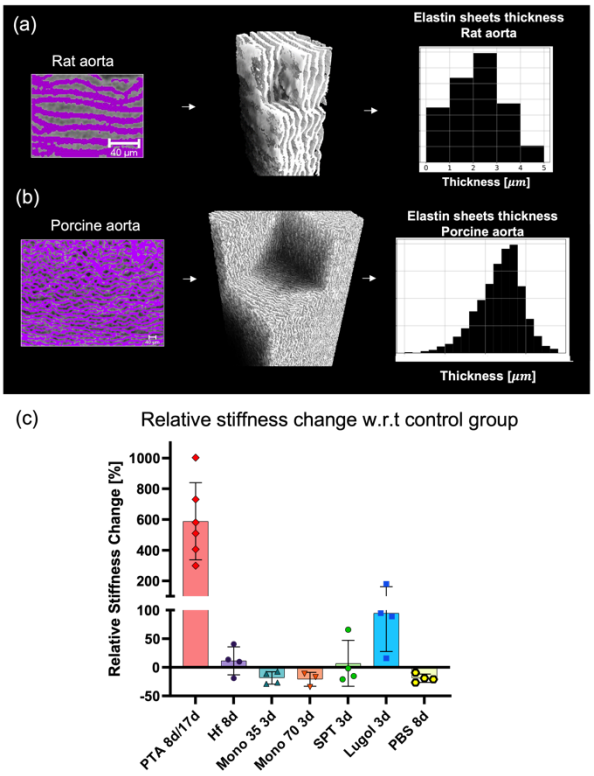


Figure 1: Comparison between rat aorta (a) and porcine aorta (b) of CECT 2D cross-section with region of interest in the media, where the elastic lamellae are segmented in purple (left), CECT-based 3D volume of the elastin lamella (middle) and their thickness distribution (right). Relative tangential stiffness change with respect to control group for the different conditions (6 CESAs & 2 Controls) from uniaxial tensile tests in circumferential direction (c). Put in full the names under the graph