Cryogenic contrast-enhanced microCT (cryo-CECT) enables 3D quantitative histopathology of soft biological tissues

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Introduction

Given the close structure-function relationships in biological tissues[1], the importance of histo(patho)logy in understanding the functioning of healthy and pathological tissues cannot be understated. Moreover, it allows to evaluate the impact of possible treatments, including tissue engineering (TE)-based approaches, on the tissue microstructure (i.e., the structural organization at the microscale). However, classical 2D histological techniques only partially reveal the spatially complex 3D microstructure of biological tissues[2]. In this regard, X-ray based 3D histology, using contrast-enhanced microCT (CECT), can provide important complementary microstructural information [3,4]. Here, we present an extension to conventional CECT, termed Cryogenic Contrast-Enhanced MicroCT (cryo-CECT), to perform quantitative 3D histo(patho)logy of tissues [5]. Cryo-CECT combines sample staining, using an X-ray contrast-enhancing staining agent (CESA), with freezing at the optimal freezing rate. The sample is then imaged by microCT in the frozen state using an in-house developed in-situ cryo-stage. We demonstrated that cryo-CECT enables 3D visualization and structural analysis of individual tissue constituents, such as muscle and collagen fibers.

Results and discussion

Cryo-CECT was first optimized in terms of CESA and freezing rate using bovine muscle tissue (Fig. 1 a-d). We then applied cryo-CECT on murine hearts that underwent transverse aortic constriction (TAC) surgery, a well-established experimental model for pressure overload-induced cardiac hypertrophy. Cryo-CECT allowed to analyze, in an unprecedented manner, the orientation and diameter of the individual muscle fibers in the entire heart (Fig. 1 e), as well as the 3D localization of fibrotic regions within the myocardial layers (Fig. 1 f). With this analysis, we demonstrated the added value and complimentary of cryo-CECT to conventional histopathological tools.

Conclusion

In this study, we presented a novel approach to CECT, termed cryo-CECT, which enables nondestructive 3D histo(patho)logy of various individual soft tissue constituents by imaging the stained sample in its frozen state. The application of cryo-CECT to murine hearts that underwent TAC surgery demonstrated the histopathological potential and added value of this new technique.

References

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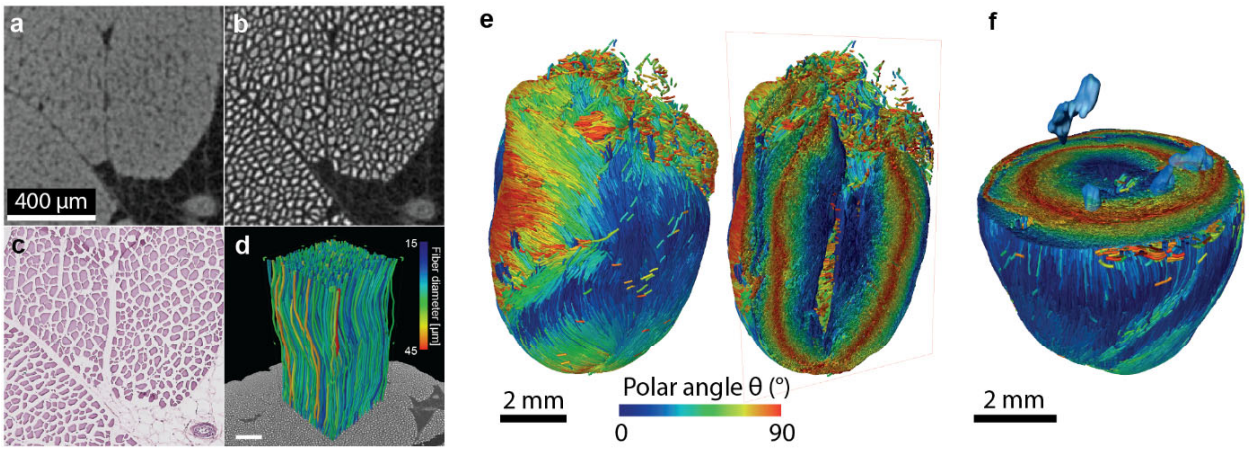

Fig. 1 | Cryo-CECT allows 3D histo(patho)logical assessment of skeletal and cardiac muscle tissues. a-b, MicroCT images of the same bovine muscle sample stained with Hf-WD POM, acquired with conventional CECT (a) and with cryo-CECT (b). **c,** Conventional 2D histological section (H&E staining) matching the previous microCT images. **d,** 3D rendering of the fiber model based on the cryo-CECT dataset of (b), in which the diameter of each fiber is indicated by the color scale. Scale = 500 µm. **e,** 3D spatial graph of the cardiac muscle fiber orientation a TAC heart. Fiber orientation is represented by the inclination, or polar angle θ and is indicated by the color scale. **f,** 3D spatial graph of the cardiac muscle fiber orientation of a TAC heart, showing the location of the regions that were severely affected by interstitial fibrosis (blue volumes).