HIGH-RESOLUTION CONTRAST-ENHANCED MICRO-CT FOR SPATIAL ASSESSMENT OF BIOLOGICAL TISSUES

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Background

As biological tissues have a complex architecture and a spatial heterogeneity, measurements made in 2D only partially reveal their full 3D morphology and interconnectivity. Indeed, conventional and even digital histology are largely unsatisfactory for spatial assessment of biological tissues, because the restricted sectioning orientation and the limited depth resolution only partially reveal their full 3D morphology, but also because they lack reproducibility on a large scale (sample destruction). In this overview, the potential and added value compared to standard 2D imaging techniques of contrast-enhanced X-ray microfocus computed tomography (CE-CT) are presented. CE-CT is a very recent development in the micro-CT imaging field and allows **quantitative virtual 3D histology** of both soft and mineralized tissues.

Prof. Greet Kerckhofs has been recently appointed as Associate Professor at the Institute of Mechanics, Materials & Civil Engineering (UCLouvain, Belgium) running the Biomechanics Lab, and as Visiting Professor at the Dept. of Materials Engineering (KU Leuven, Belgium). She is Scientific Committee member of Prometheus, the Division of Skeletal Tissue Engineering (KU Leuven). She obtained her PhD in Materials Engineering (KU Leuven) in 2009, and was a postdoc at the Université de Liège (2012-2014) and the KU Leuven (2009-2011; 2014-2017). Her present research mainly focuses on 'Quantitative virtual 3D Histology using Contrast-Enhanced CT'. She is author of 44 peer- reviewed publications, two book chapter and more than 100 contributions to International and National Conferences. She has an h-index of 17, with more than 1300 citations.

Development of novel contrast agents

Over the past decade, different CAs have been recently reported for CE-CT of specific soft tissues [1]. However, both the CA staining protocol and the image acquisition setup should be non-destructive, having no effect on the tissue integrity. As most of the currently applied CAs are destructive (acidic, toxic, dehydrating), in our research we focus on the development of non-invasive tissue-specific CAs. Recently, we reported the simultaneous visualization of mineralized and soft structures within bones utilizing an in-house Hafnium-substituted Wells-Dawson polyoxometalate (Hf-WD POM) [2]. Hf-WD POM allowed visualizing, apart from bone, the bone marrow adipocytes at the single cell level, as well as the vascular network allowing full 3D blood vessel network assessment (i.e. branching analysis and spatial distribution).

In a follow-up study, we explored whether similar POM formulations could also be efficient CAs for soft tissue visualization [3]. We screened different POM formulations, including the much less expensive precursors of the metal-substituted Hf-WD POM, for their potential as CE-CT CA. Based on their staining capacity and speed of diffusion, as well as on their fate after tissue staining experiments, we concluded that monolacunary POMs are highly suitable staining candidates for non-invasive CE-CT imaging of soft tissues, with a similar contrast enhancement as that of Hf-WD POM and phosphotungstic acid, an invasive CA that has been typically used in the past.

Relevant applications

Because of their capacity to bind electrostatically to collagen and connective tissues, as well as to blood, we have successfully used the POMs for several applications: developmental changes of murine placenta and embryo [4], effect of an anti-angiogenic drug on tumour angiogenesis [5], quantitative analysis of the fibre orientation in tendons and the bone-tendon interface (unpublished data), and many others.

Future directions

CE-CT is an important and innovative 3D imaging technology to get a better understanding of the complex mechanisms behind tissue formation and regeneration, as well to better understand the effect or pathologies on tissue architecture and hence function. In future research, we will focus on the further development and validation of novel, tissue-specific CE-CT CAs in a wide range of applications, and on the combination with deep learning image analysis for improved quantitative 3D analysis of the CE-CT datasets.

References

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