Exploring Polyoxometalates as Non-invasive Staining Agents for Contrast-Enhanced Microfocus Computed Tomography of Biological Tissues

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Despite being destructive, conventional histology still is the gold standard for structural analysis of biological tissues. And although histology is limited by 2D sections of a 3D object, its success is manifested in its high discriminative power. X-ray microfocus computed tomography (microCT) has proven to provide full 3D morphological analysis of mineralized biological samples in many biomedical fields. Due to the intrinsic low contrast of soft tissues, contrast-enhancing staining agents (CESAs) are necessary to allow differentiation. In a previous study, we showed the potential of a Hafnium-substituted Wells-Dawson polyoxometalate (Hf-WD POM) to simultaneously visualize mineralized bone, marrow vascularization and adipocytes.[1] In this study, other polyoxometalate (POM) species have been examined for their potential as soft tissue CESAs for contrast-enhanced microCT (CE-CT) imaging.[2] Four Wells-Dawson POMs, differing in structure and overall charge, were used to stain murine long bones and kidneys. Their staining potential as well as diffusion rate were compared to that of Hf-WD POM and phosphotungstic acid (PTA), a frequently used, but invasive CESA. Soft tissues stained with Mono-WD POM showed a similar contrast enhancement as that of Hf-WD POM and PTA. Moreover, Mono-WD POM is less invasive and shows a better diffusion than PTA, and its synthesis requires less efforts (*i.e.* time and cost) than metal-substituted POMs. Finally, the solubility of Mono-WD POM was improved by adding LiCl to the staining solution, hence enhancing the contrast of soft tissues.

[1] Kerckhofs, G., *et al.*, *Biomaterials* **2018**, 159, 1-12. [2] De Bournonville, S.*; Vangrunderbeeck, S.*, *et al.*, **2019**, *Manuscript in preparation*. *Joint first authors