Spatial Localization of Vitamin D metabolites in Mouse Kidney by Mass Spectrometry Imaging

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Introduction

- Vitamin D plays a major role in biological functions such as bone health and immune health[1,2] and as a potential treatment for diseases including cancer[3]
- Metabolites of vitamin D possess poor ionization efficiency that can be enhanced by chemical derivatisation (CD)
- Mass spectrometry imaging (MSI) may provide information about tissue-specific metabolism

Aims of the study

- To develop an on-tissue chemical derivatisation (OTCD) method
- To identify and assess intratissue metabolism

Materials and Methods

- Mice tissue sections were cryosectioned at 12µm and thaw mounted onto I.T.O. slides for MSI experiments
- PTAD, DMEQ-TAD and Amplifex were all screened for OTCD ionization efficiency using labelled VitD metabolite
- Artistic airbrush application and Bruker's ImagePrep were assessed for reliable derivatisation reagent deposition
- MALDI-MSI was performed on 9.4T SolariX with CASI and FlexImaging. DESI-MSI was performed on 2D DESI source with Waters Xevo G2-XS mass spectrometer. Both in positive ion mode
- Confirmatory LC-MS/MS was carried out on kidney homogenate on a ACQUITY UPLC with an ABSciex 6500 QTrap

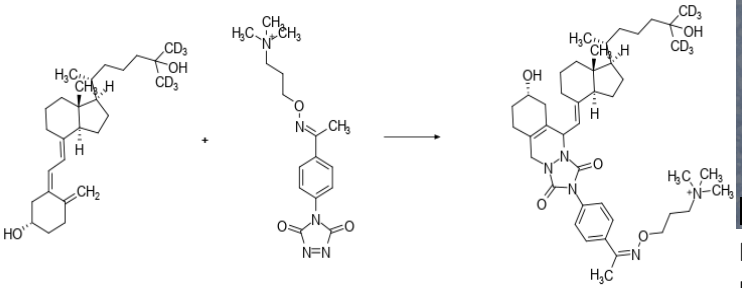

Figure 1. 1,25-VitD$_3$-Ampiflex derivatization reaction

Results

1. MALDI and DESI comparisons (Tissue spotting experiments)

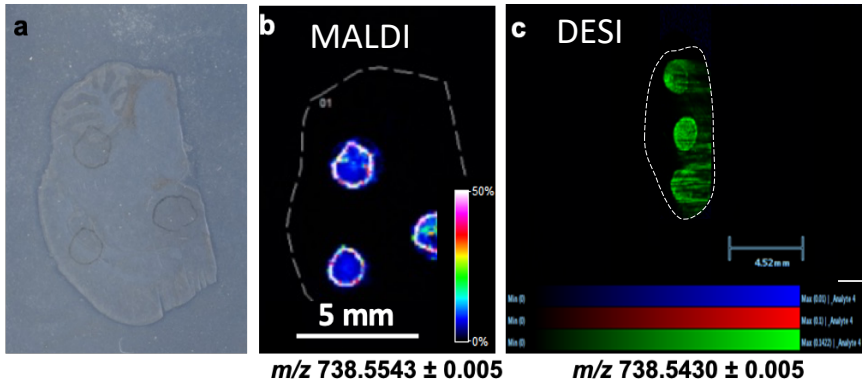

Figure 2: Tissue spotting experiments using a d_6-25-(OH)-D_3 analysed by MALDI-FT-ICR-MSI (b) and DESI-qTOF-MSI (c). MALDI-MSI demonstrated best ion production yields with less analyte diffusion. Amplifex was the best candidate, producing the most abundant signal with best S/N ratio

3. MALDI-MSI of endogenous Vit D metabolites

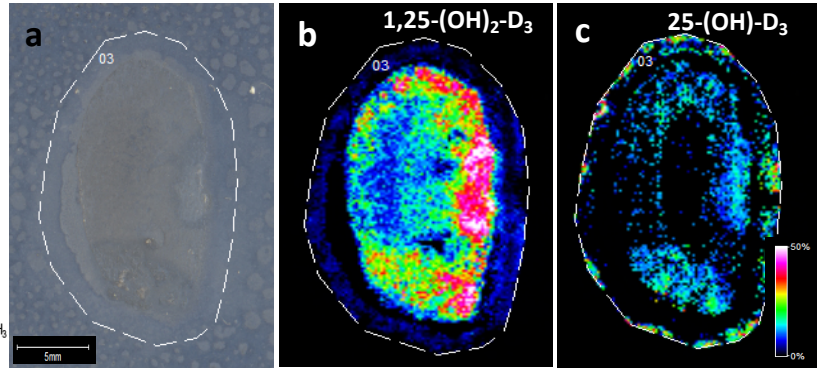

Figure 4. Molecular distribution of endogenous VitD metabolites in mouse kidneys (b) 1,25-dihydroxyvitamin D_3 (*m/z* 748.5001) and (c) 25-hydroxyvitamin D_3 (*m/z* 732.5058) using Amplifex OTCD-MALDI-MSI

2. Derivatization reagent application (Manual Vs Automated)

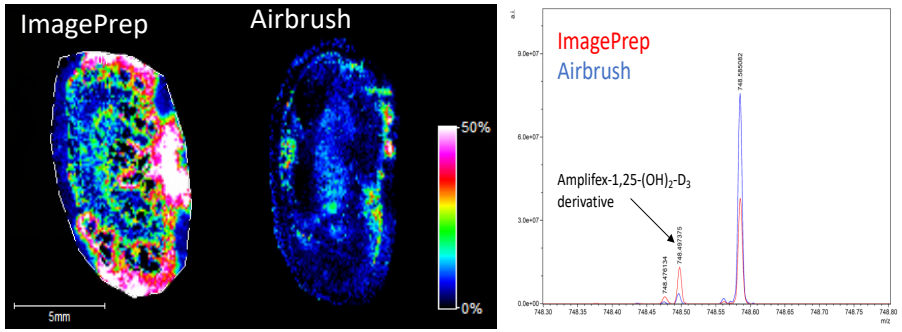

Figure 3. A customised ImagePrep method was assessed and compared to manual airbrush. The ImagePrep outperformed the airbrush in tissue-to-tissue reproducibility, S/N ratios and analyte diffusion. The ImagePrep also introduces an inert environment for the reaction to occur for a one hour long reaction time.

Conclusions

- Spatial distribution on Vitamin D metabolites can be achieved on tissue by employing OTCD-MALDI-MSI methods by using the Amplifex-diene reagent.
- Best reproducible results can be obtained with the use of automated CD application devices, such as the Bruker ImagePrep.

References

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[3]Smith K.W; Thompson P.D; Rodriguez E.P; Mackay C.L.; Cobice D.F. *Biochemical and biophysical research communications. 2019, 519(3): 579-84.*