High resolution mass spectrometry imaging for pharmaceutical and clinical applications

Impact

The work presented in this thesis has demonstrated, next to the scientific impact, also a societal impact and the results of this are tangible in that these are finding their way onto the market and into society. Most notably this is expressed in the work on the sublimator described in chapter 2.

The initial development of the sublimation device was a collaboration between M4I, IDEE and Brightlands. The potential of the first prototype of the developed and evaluated, sublimator led to an extended collaboration between M4I and IDEE to further improve the sublimation device. This collaboration resulted in the device presented in chapter 2 offering a new, easy-to-use, reliable and reproducible sample preparation device for MALDI-MSI.

In the development process two patents for the device were filed and this in combination with the performance of the device led to the licensing of the device to a commercial party, HTX technologies. In conjunction with the licensing, the sublimator contributed in attracting HTX technologies to set up a European branch of HTX on the Maastricht Brightlands campus, HTX imaging BV. The Sublimator was first presented by HTX at the ASMS 2018 conference and is actively marketed on their website and sales are generated. Furthermore, HTX Imaging BV has set-up a manufacturing at a facility in the Limburg region resulting in the Sublimator having a direct economic contribution, and importantly, a major part of this economic contribution is retained within the local region providing labour in the region. Furthermore, an extended collaboration agreement between HTX, IDEE, and M4I was made for the further development and evaluation of matrix application devices including not only the sublimator but also further development on the matrix sprayer.

Next to the economic contribution of the sublimator, it also offers the scientific community access to a user-friendly reproducible sublimation device which consumes less matrix than other commercially available matrix sublimation devices. This contributes to the sustainable chemistry goals defined worldwide.

The ambition to mature MSI into a clinically compatible technology requires efforts on many fronts and from different people. Next to improvements in the analytical systems and their

application in large cohort studies to find relevant (combinations of) biomarkers, it is vital to have workflows that are compatible with clinical diagnostics, both from a time/logistics point of view and from an economical point of view. The development of the protocols described in this thesis for rapid sample preparation as proof of concept that MSI can perform tissue analysis within the required time frame is an important step. Moreover, EU-patent 18192007.5 describes the use of precoated matrix cartridges developed for the sublimator, which was simulated by spray-coating the matrix holder as well as a glass slide that was placed in the matrix holder of the sublimator. This has great potential for clinical use since it offers a level of consistency and potential for full automation. Furthermore, it would reduce the need of manual handling of potentially toxic MALDI matrices offering a further advantage from a health and safety perspective.

The use of quantitative MSI was essential in demonstrating that a significant amount of drug can remain in the lumen of the intestine, despite intensive flushing of the intestine with a wash solution, leading to an overestimation of tissue drug content when intestine homogenates are analysed by conventional LC-MS. This clearly demonstrate the benefit of MSI as complementary technique for pharmaceutical applications. This does not discount the use of LC-MS for evaluation of tissue drug content, however, in particular for the intestine, it is critical to be aware of the risk of flushing of tissue to be insufficient to completely wash away the drugs from cavities such as the intestinal lumen, so that additional steps can be taken to address this factor, e.g. the use of laser capture microdissection or cutting the intestine open to allow for a more thorough flushing of the intestines prior to LC-MS analysis.

The 3D-visualization of drug intensity described in chapter 3 is an outstanding visualization tool to determine drug uptake processes. This is not only limited to drug uptake in the intestine but could for example also be used to visualize drug penetration in tumours. This is of great added value as drug uptake in tumour cores is often found to be challenging and can lead to treatment resistance and tumour persistence/recurrence.

The value of quantitative MSI is also expressed in the emergence of commercial software packages targeted at quantitative MSI, such as msIQuant and MassImager. Although the

quantitative method described in this thesis is largely a manual process, the current state of image recognition software could potentially automate the region selection and in that way offers potential to be incorporated into quantitative imaging software packages. In addition, an incorporation of tissue type specific quantitation methods in conjunction with tissue annotation and the 3D-visualization would allow for a visualization of concentrations rather than relative intensities, providing an even more accurate representation of drug absorption/penetration profiles

Sensitivity is a limiting factor in (quantitative) analytical methods of low sample amounts, i.e. high spatial resolution in MSI. The use of CASI as proposed in chapter 3 is a useful way to enhance sensitivity for pharmaceutical analyses from the mass analyser side. Moreover, the isolation window used for CASI measurements can be tuned to potentially include the analyses of drug metabolites which can be of great benefit to PKPD studies. Alternatively, with the recent commercialization of MALDI-2, MALDI-2 offers an excellent alternative to enhance sensitivity.

The use of MSI in clinical diagnostics, such as e.g. oncology, where treatment is often based on biomarker information or more general indicators, offers a huge potential for e.g. treatment prediction. In patients suffering from CCA, a low survival rate is largely due to late diagnosis and a limited understanding compared to other more studies tumours such as breast or colon cancer which due to improved screening procedures are often detected in an earlier stage and due to their high prevalence have been more extensively studied. Therefore, additional knowledge on the mechanisms and progression off the disease is vital, eventually leading to a more thorough subclassification of CCA such as is the case with for example Her2 positive versus negative breast cancer with different treatment strategies. Sulfatides could be playing an important role in the functioning of bile duct and are therefore an interesting target for the study of cholangiocarcinoma. Since, in liver tissue, sulfatides are a distinctive feature of bile ducts versus other liver tissue, sulfatides serve as a potential marker for CCA. Although MSI is currently not at the level of clinical diagnostics there is potential for it to serve as a tool to rapidly distinguish CCA tumour from “healthy” tissue, especially in combination with high throughput methods such as those developed in this thesis. In addition, the detected profiles also have the potential to be used in conjunction

with intra-operative MS techniques such as REIMS. These intra-operative techniques directly analyse the sample *in-vivo,* in the case of REIMS by analysing the smoke created by the electrosurgical knife. Notwithstanding the current state of MSI for clinical use, the high abundance of sulfatides in CCA tumour tissue versus liver parenchyma could also function as diagnostic markers of tumour using LC-MS or other sulfatide targeted bioassays of liver biopsies. This allows MSI to serve as an initial tool to characterize tumours versus healthy tissue to aid the discovery of markers. In the most ideal case, sulfatides could be assessed as biomarkers in biofluid based assays (e.g. plasma, urine). LC-MS based assays for plasma sulfatides already exist and are also evaluated for other disorders.

Although in this case the research question and hypothesis focussed specifically on the role of sulfatides in CCA it should not be forgotten that the data generated with MSI for this study, contains a broad class of other molecules detected as well and therefore the dataset can also still function for further exploratory, non-hypothesis driven, evaluation of the different tumours evaluated in this study. High-resolution data is especially beneficial to this respect as it allows a better tentative assignment of the molecules and as such the potential pathways involved in CCA. Lipid composition such as different degrees in hydroxylation and saturation have an impact on the tumour behaviour and the efficacy of tumour treatment. The initial study of sulfatides in CCA could provide a basis for further evaluating the role of sulfatide composition in tumour treatment efficacy.