Clinical application of mass spectrometry imaging for analysis of bone and cartilage

Chapter 8: Impact paragraph

8.1 Scientific impact

The scientific impact of the work described in this thesis can be divided between the impact of the developments for sample preparation for matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) and the development of a 3D imaging setup using laser-assisted rapid evaporative ionization mass spectrometry (LA-REIMS), which will be discussed separately.

The sample preparation protocols developed in this thesis for MALDI-MSI allow for the analysis of new tissue types, namely bone and fracture hematoma, that were limitedly analyzed with this technique before. In the case of bone tissue, the developed embedding and sectioning protocol allows for the sectioning of undecalcified bone tissue with limited fragmentation. The sectioning of bone tissue is complicated by the different structures present. The optimized sample preparation protocol for mouse, rat, and human bones as well as a targeted forensic and untargeted preclinical application were published in Analytical and Bioanalytical Chemistry and was presented as a lecture and as a poster during a 24 hours IMSI (International Mass Spectrometry Imaging) conference. Bone tissue can be analyzed with MALDI-MSI using this protocol allowing for a broad range of potential applications, for example, in preclinical and biomedical research, forensic research, and paleontology. In preclinical research, MALDI-MSI analysis of undecalcified bone tissue can be used to improve molecular understanding of different healing conditions of the skeletal system. One of these topics was the research into the molecular pathways during bone fracture healing and the impaired pathways in non-union development, which was the main protocol development target. The animal study into the effects of citrulline supplementation during fracture healing described in this thesis is an example of an application of the developed MALDI-MSI protocol. Other applications related to bone diseases, like osteoporosis and bone cancer, are now possible as well. In biomedical research, the sample preparation protocol will find innovative applications, for example, in the field of tissue engineering for the analysis of fabricated bone with MALDI-MSI. In forensic research, it can be applied for the detection of drugs and other compounds that might have been related to someone's death, like methadone and its metabolite EDDP. The potential application in the field of paleontology can be to study the different components present in fossils.

In the case of fracture hematoma, the optimized washing method allows for the analysis of the tissue with MALDI-MSI. The washing method is essential to reduce the ion suppression caused by heme presented in the blood of the fracture hematoma. In addition, this was the first time that fracture hematoma was

analyzed with MALDI-MSI. The optimization of the washing methods as well as preliminary results about lipid patterns at different time points after bone fracture were published in Frontiers in Chemistry and were presented as a poster during a TERMIS (Tissue Engineering and Regenerative Medicine International Society) conference, an ECTES (European Congress of Trauma and Emergency Surgery) conference, an EFFORT (European Federation of National Associations of Orthopedics and Traumatology) conference, and an IMSC (International Mass Spectrometry Conference) showing the broader impact of this research. The analysis of fracture hematoma with MALDI-MSI will mainly be applied in preclinical and biomedical research, as bone fracture hematoma are specific for bone fractures, although hematoma are formed after injury. The MALDI-MSI analysis of fracture hematoma can be used to improve molecular understanding of bone fracture healing and non-union development. The general results obtained during the optimization of the washing protocol can be extrapolated to other tissues containing substantial amounts of blood. The washing method will need to be tested and optimized per tissue type, due to structural differences between tissues.

The 3D MS Scanner is developed to deploy LA-REIMS for the imaging of uneven sample surfaces. The setup is one of the few techniques that has been developed for 3D imaging of sample surfaces with height differences in terms of millimeters. The setup was not patented, as similar techniques were already developed in parallel by other research groups. This imaging setup is the first one to use a surgical CO_2 laser in combination with REIMS. Furthermore, it is the first ambient mass spectrometry 3D imaging performed on the bone from marrowbone and cartilage from a human femoral head resulting in different molecular distributions on these surfaces. LA-REIMS is one of the few ambient mass spectrometry techniques that can be applied to these tissue types. The 3D MS Scanner and the obtained results with this setup were published in the Journal of the American Society for Mass Spectrometry and orally presented at a MSACL (Mass Spectrometry & Advances in the Clinical Lab) conference. The 3D MS Scanner can be used to visualize molecular distributions on sample surfaces with height differences by applying 3D imaging, as indicated. This allows for the extensive analysis of surfaces of a wide range of objects. The fields of application can be (pre)clinical and biomedical research, the food industry, material sciences, and many others. In (pre)clinical and biomedical applications, the surfaces of whole bones and joints could be analyzed to explore the molecular distributions. This can be used to improve molecular understanding of cartilage damage and repair and the impaired pathways in osteoarthritis (OA) and post-traumatic osteoarthritis (PTOA) development. This data can be used to build more accurate recognition models, for example, for the classification of

normal versus impaired healing. These models can be used for *in vivo* recognition with precise *in vivo* sampling using LA-REIMS. This is just one example of a potential (pre)clinical and biomedical application, but many other tissue types could be analyzed with this setup. Furthermore, the 3D MS Scanner could be used in the food industry, for example, to study the distribution of compounds (pesticides, waxes, etc.) on the surfaces of these foods or to explore the effects and spread of parasites, bacteria, and fungi on the surface of the food, such as fruits and vegetables. Another example application of the 3D MS Scanner is its use to analyze the surfaces of developed materials to evaluate their chemical homogeneity in the field of material sciences. In general, almost all objects could be analyzed in the 3D MS Scanner with the application of LA-REIMS as long as they fulfill four requirements: 1) the object has to maintain its shape while it is being moved during the topographical scan and the mass spectrometry acquisition; 2) ablation of the material of the object should be possible with a CO_2 laser; 3) ionization of the ablated molecules should be possible with REIMS; and 4) the resulting ablated smoke and molecules should not contaminate the source or mass spectrometer.

8.2 Social impact

In general, the end goal of (pre)clinical research is to apply research in such a way that it can help to improve health care and the patient's quality of life. Possible improvements are a progression in understanding of the disease, formulation of new treatment options, earlier or more accurate diagnosis, or prediction of outcome. Currently, the burden of non-union and PTOA development is high for the patients. This is due to the amount of time between bone fracture or cartilage damage and diagnosis as well as the long and/or intensive treatments and revalidation times, which causes a lower quality of life for the patients during these periods. The results as described in this thesis are preliminary and cannot directly be applied in the clinical environment without further validation through future research and clinical trials. These applications do have the potential to evolve from research to earlier prediction of outcome in the near future. In addition, the citrulline supplementation study showed enhancement of fracture healing in a rat model, which could be further explored in impaired healing and is a potential clinical target. The developed MALDI-MSI methodologies as well as the 3D imaging approach for LA-REIMS can be used to study the impaired molecular pathways involved in the development of non-unions and PTOA. This can improve molecular understanding by defining which pathways are essential for bone fracture healing and cartilage repair. In addition, the different molecular profiles between normal and impaired healing can be explored. These profiles can be used to build recognition models for early diagnosis and prediction of outcome. This early

prediction of the outcome can be done either *in vivo* using LA-REIMS for near-real time diagnosis during operation, or *ex vivo* by applying LA-REIMS or MALDI-MSI on a sample taken during surgery. The earlier prediction of outcome allows a clinician to adapt or start additional treatment before the onset of the symptoms related to non-union and PTOA development and possibly prevents the development of non-union or PTOA resulting in improved quality of life for the patient and reducing the burden of the disease.

This research emphasizes the importance of collaborations between the research institutes and clinical practice. This is specifically the case in (pre)clinical and biomedical research, as the researchers in these fields try to answer clinically relevant questions. The first important and impactful aspect of collaboration is to define an unmet clinical need, which can be either a more fundamental or a more applied question. The fundamental questions answered using mass spectrometry are molecular by nature and can be, for example, to understand the importance of one specific molecule in a certain pathway or disease, or to find a molecular profile that can be used for the classification between healthy and disease as well as different disease types or stages. All these questions are related to the improvement of the molecular understanding of health and disease. Mass spectrometry targets the discovery of molecular profiles that can be used for early diagnosis and prediction of outcome, which would allow for earlier adaption of treatment if necessary. Samples of different disease stages or classes as well as healthy tissue is necessary to answer these questions. Therefore, the second aspect of the collaboration is for the clinic to provide part of the samples necessary for the research if possible. These clinical samples are of importance to finding molecular profiles and test prediction models, as not all research can be based on animal models alone. The last important aspect is the exchange of knowledge between research institutes and the clinic. Often, all the different possibilities and techniques in the research institutes are not well known to the clinicians. It is important to ensure they get familiar with the possibilities and understand how the techniques can be applied. The other way around, the researchers work hand in hand with the clinicians to understand not only their unmet needs, but also to learn from what they already know and what the hurdles are when applying such a technology in “real life”. The collaboration between research institutes and clinical practice is essential to incorporate mass spectrometry techniques like LA-REIMS and potentially MALDI-MSI into patient care.

Besides collaborations between research institutes and the clinic, the collaborations between different research institutes are also of importance.

Sharing knowledge about all the different aspects of research boosts the research performed at the collaborating institutes. This is accomplished via CORE lab (collaborative open research and education) within M4i, which, among others, allows other research groups to use the facilities at M4i. I had the pleasure of experiencing this type of collaboration myself during my month-long visit to Martina Marchetti-Deschmann's research group at the TU Wien (Vienna, Austria). I learned a lot about the sample preparation protocol developed by a research group member (Anastasiya Svirkova) and another guest visitor (Michiel Vandenbosch) during this visit. Without this knowledge, I would not have been able to get the MALDI-MSI results on bone tissue as presented in this thesis. This visit also resulted in a shared publication between the research institutes, namely the research shown in Chapter 3. In terms of personal development, this visit taught me that I can adapt to a different research group and its dynamics. In addition, I developed my experimental and data analysis skills further during this visit. Of course, I also used my time in Vienna to explore many of the cultural sights.

Performing a Ph.D. project and the related research is of course a personal investment as well. The obvious acquired skills are the knowledge I acquired about different sample preparation and mass spectrometry techniques. I learned a lot about data analysis and visualization in general as well as specifically for mass spectrometry data. My instrumental knowledge increased as well, especially because of all the troubleshooting on different instruments that I had to perform over the past years. In addition, I developed numerous soft skills further, for example, scientific writing and presenting. It was confirmed for me that I work in an organized and structured manner throughout my project, although I should be on the lookout to not let this slow me down. Furthermore, I developed my communication skills during the past years. Not only in the form of scientific publications and presentations at international conferences, but also in the communication with different types of people. Specifically, the collaboration between M4i and MUMC+, which is the basis of this research, challenged me to explain my research, for example, to surgeons. This collaboration is important to me, as the potential direct clinical implications and applications of the project drove me. In addition, it made it possible for me to visit the operating room during which I could see the challenges surgeons are facing. All of the things I learned during the past years have turned me into the person I am now and will impact the rest of my carrier.

8.3 Economic impact

The future applications of the developments described in this thesis are, in short, the improvement of molecular understanding in the development of non-unions and PTOA as well as the early prediction of outcome based on these different molecular profiles. In addition, the effect of citrulline supplementation on the lipid and protein profiles during fracture healing can be used for improved molecular understanding as well as a potential clinical target. Improved molecular understanding could result in the development of new or more efficient treatments to prevent or cure impaired healing. These developments can be interesting for pharmaceutical companies, as they can research, fabricate, and distribute these new treatment options. In addition, the potential early prediction of outcome might require additional developments that different companies can contribute to, in the mass spectrometry field as well as beyond. Mass spectrometers need to be further developed such that they can be placed in the operating room without being affected by the cleaning processes taking place there and without taking too much space in case of *in vivo* prediction of outcome using LA-REIMS. In addition, companies might contribute to the development of the surgical CO_2 laser. This laser was selected, as it is already clinically approved, but, for example, future improvements could be made to the spot size of the laser to reduce the tissue damage. For the *ex vivo* application of MALDI-MSI, companies could develop sample preparation kits that would allow for quicker and/or easier sample preparation of bone tissue and fracture hematoma. Another possibility for an investment of companies or a spin-off company is offering sample analysis to hospitals. In this case, the distinguishing profiles for normal and impaired healing should have been defined. A company could in that case do the sample preparation and analysis and provide the surgeon with the information. This could be applied to the *ex vivo* application of MALDI-MSI or LA-REIMS. Another option for a spin-off company is to further advance the developed 3D MS Scanner to sell it as an ambient 3D imaging technique. For example, other types of lasers or probes could be explored or the sample types could be broadened with different types of sample holders. These are just a few of the examples in which companies could invest based on the research performed in this thesis.

Besides the potential investment options for companies, the potential development of new treatments and the early prediction of outcome can both contribute to shorter treatment times and, thereby they will reduce health care costs. A specific example of this is the potential fracture healing enhancing effect of citrulline supplementation after bone fracture, as shown in this thesis. Currently, the treatments for non-union and PTOA are long and expensive and, therefore,

these treatment costs are an economical burden. Contributing to the high treatment costs is the long duration until diagnosis. Overall, the research presented in this thesis positively impacts the costs related to non-union and PTOA development in health care.