Biomarkers in cartilage repair and osteoarthritis

Chapter 8

Impact

Social and economic impact

Understanding the molecular roots of a disease could advance (personalized) treatment, improve a patient's quality of life, and contribute to better (public) health care. A biomarker (panel) for cartilage repair patient outcome and early osteoarthritis (OA) development would improve accurate diagnosis, surgical decision making, and facilitate personalized treatment, inhibiting OA development and improving a patient's quality of life by increasing its ability to move. This will eventually lead to a reduction in OA-related comorbidities, a reduction in total knee arthroplasty (TKA) revision surgery accompanied with a reduction in surgery-related infections, and a reduction in health costs. However, it will take years before these benefits are accessible for the patient, as scientific studies need to be converted into clinical trials and money needs to be invested to make it applicable for the patient.

Unlike for example in cardiology, where asymptomatic patients are treated according to e.g. blood pressure or blood lipid levels, there is no proper measurement control in the diagnosis of pre-, early, or moderate OA. When OA is diagnosed early in its development, preventive measures, such as an already used anti-inflammatory drug like celecoxib, with a chondroprotective potential, could contribute to this delay in OA development. Additional research on the effect of celecoxib on the pathways involved in OA development could lead to a better understanding of the molecular changes happening in OA and facilitate the development of (novel) therapeutic targets and treatments. Potential injection with disease modifying OA drugs (DMOADs) in an early phase of the disease is of interest, however, the outcome might only be visible on MRI (structural changes), rather than improving quality of life by e.g. reducing pain, where pain is often not existing in early stages of disease. Therefore, at this moment, the FDA (Food and Drug Administration) dictates that (injectable) drugs should act on both pain as well as structural changes.

Early diagnosis of OA would give the opportunity to intervene early in the disease development and postpone TKA and revision surgery. MALDI-MSI can give us a better understanding of the molecular changes, as well as the molecular distributions of OA development. Knowledge on the molecular profiles can be applied to point-of-care *in situ* analysis techniques such as REIMS for the development of pattern recognition models for early diagnosis of OA or prediction models for cartilage repair. According to the results acquired, a clinician can adjust treatment to the patient's profile, start OA treatment early in its development, and possibly prevent OA from developing further. This would lead to a reduction in TKA

surgeries, TKA revision surgeries, and health care costs, while improving a patient's quality of life and public health. The biggest risk of losing an implant are stress shielding – a reduction in bone density – or bacterial infection. Especially prosthetic joint infections are difficult to treat as patients are often too weak for additional surgery and antibiotics alone do not eradicate the infection, leading to bacterial resistance. The FDA has acknowledged this problem and pays special attention to the majority of joint-preserving treatments.

In this research, we address the importance of data sharing, as well as the importance of collaborations between research institutes and clinical facilities. In the field of biomedical research, studies are conducted to find answers to clinical questions and improve patient care. The fundamental research conducted throughout this Thesis provides us with results, which can lead to a better understanding of disease development and pathways involved. Working closely together with a university medical center, the lines between fundamental findings in the laboratory and its application in the clinic are kept close. Here, the clinic provided us with the essential patient samples for the identification of molecular pathways involved in the closest human situation available. Knowledge should not only be shared between research institute and hospital, but also between research institutes. Providing other researchers with information on protocol development, application, and data acquirement (even if it is not working) is of great importance as usually only small optimization steps (dependent on the tissue type, materials, and techniques available) have to be taken prior to utilization. This would eventually save time, reduce workload, and experimental costs.

The biomedical research conducted in this Thesis would not have been possible without the financial funding of a variety of instances and companies. Not only have we been collaborating between the Laboratory for Experimental Orthopedics, the department of Orthopedic Surgery of the MUMC+ (Joint-Preserving clinic), and the Maastricht Multimodal molecular imaging institute (M4i), our data has also been presented to the instances and companies financially involved. Collaborations between research facilities and companies makes it possible to get new developments patented, further developed, and produced for clinical implementation. In addition, changes in design of the instruments used (e.g. for application in the clinic), can be made by the owner company who is invested in a type of treatment or a novel method. Whereas companies have the best interest in helping the patient, developing new treatment strategies, incurred costs from many years of research have to be repaid, bringing potential high health care costs. The patient and provider (e.g. the surgeon or hospital) is caught between the incentive

of articles showing promising results and patents of products on the other hand. In this Thesis we showed that with a close collaboration between the patient (having a problem), the surgeon (bringing the question), researchers (providing the research and results), funds, and companies (providing money), this gap might be closed. However, the risk vs benefit, as well as data vs ownership coming with this type of research are still under debate.

Scientific impact

Early detection of OA development is of great importance to intervene in its progression and outcome by treating the early stages of the disease. Biomarkers might contribute to the development of novel therapeutic treatment options. In this Thesis, we provide further knowledge on the (lipid and protein profile of the) infrapatellar fat pad (IPFP) and acknowledge its function as biochemical organ in maintaining (healthy) joint homeostasis. Fat pads are present in every joint and are highly available and accessible for research or as biopsy target. This in contrast to the widely studied synovial fluid, which is not easy to harvest in a healthy, pre-, or early OA patient.

Importantly, we found through our review in Chapter 2 that most likely, a combination of proteins, lipids, and metabolites would contribute to a biomarker profile for OA, rather than one single biomarker. Additionally, we are stressing the importance of the use of large patient cohorts for analysis, the use of standardized methods, and allocation of acquired information through shared and open databases. These statements should be taken into account in future scientific research while searching for novel OA biomarkers.

According to the results on celecoxib described in this Thesis, future studies should focus on the use of a single intra-articular bolus injection of celecoxib in bigger animal models, such as equine, to make implementation in the human situation possible. It is difficult to translate these findings in animal studies to the clinic, as there is no consensus on which animal model corresponds to which “theoretical” OA phenotype best. These OA phenotypes should then be connected to a treatment algorithm, as for example has been described by the Dutch Orthopedic Association (NOV) for surgical treatment of (osteo)chondral defects in the knee in 2019. The results described in this Thesis on celecoxib were presented during the International Cartilage Regeneration and Joint Preservation Society (ICRS) in 2022, where the broad impact of this research was acknowledged. Celecoxib is an example of a possible disease-modifying drug still under debate, which may benefit from the

results in **this Thesis,** as our results are not modifying, but thriving the discussion towards the benefits of celecoxib as a disease-modifying drug for OA. Our findings on potential biomarkers for cartilage repair and OA might contribute to new or additional therapeutic targets and possibly identify which patient population might benefit from the use of celecoxib in the clinic.

A variety of methods were optimized for the analysis of the IPFP lipid and protein profile in a variety of patients, with a variety of (pre-)OA pathologies. The further scientific impact of the results described in **this Thesis** is based on the methodological development of the use of matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) on the IPFP of OA and cartilage defect patients. Additionally, the differences in lipid profiles between OA and cartilage repair patients were described. Furthermore, a considerable step towards clinical implementation of mass spectrometry (MS) as a potential point-of-care technique in the form of rapid evaporative ionization MS (REIMS) was made by analyzing differences in lipid profiles of OA and cartilage defect patients, as well as differences in lipid profiles in a cartilage defect patient cohort for prediction profiling. Proteomics analysis on this same patient cohort was performed to get a better insight in the changes occurring in the IPFP of patients after a cartilage defect, as well as after surgery.

The sample preparation and MALDI-MSI analysis to measure lipid profiles in the IPFP of patients with OA or a cartilage defect has not been described before. This method allows for the sectioning of fresh-frozen IPFP and application of matrix with limited delocalization of molecules. The results contributing to this future publication have been presented during the European Orthopedic Research Society (EORS) in 2019 and 2020, as well as during the Mass Spectrometry School in Biotechnology and Medicine (MSBM) Summer School in 2019, and the LipidMaps Spring School and Tissue Engineering and Regenerative Medicine International Society (TERMIS) in 2021. By presenting our results at a variety of conferences in a variety of cities with a variety of researchers, we were able to share and discuss our knowledge on the current status of biomarkers in cartilage repair and OA. It is of great importance to share knowledge in an honest and public way to, in the end, provide the patient with the best care possible.

The method for MALDI-MSI on the IPFP has a multipurpose application, as it can be used as basis for the development of sample preparation and MALDI-MSI protocols on other fatty tissue such as abdominal fat or breast tissue. Among other applications, the use of MALDI-MSI on the IPFP has the potential to identify possible

biomarkers for cartilage regeneration, prediction models, OA development, or OA phenotyping. The IPFP is a type of tissue which is easy accessible and usually removed as waste material during cartilage repair surgery or TKA. This makes the IPFP a promising tissue type for biomarker discovery. In addition, the IPFP might be easily biopsied during out-patient clinic visits prior to surgery, as the risk at infection or other complications is relatively low. Pre-clinically, the MALDI-MSI analysis on the IPFP might contribute to a broader knowledge and better molecular understanding of the development of OA, as well as the effect of cartilage repair surgery on patient outcome.

While we slightly addressed the use of MS in the clinical setting, utilizing REIMS on the IPFP for diagnostic or prognostic purposes, this field of research needs optimization in future experiments. REIMS is a highly potential tool which can be used *in situ* in a variety of surgical applications such as cancer or cartilage repair surgery. When connected to a diathermic knife, as is already been used in the surgical theater for electrocautery cutting, no big alterations have to be made for implementation in the clinical setting. This Thesis describes the first application of the use of REIMS, connected to a diathermic knife, on the IPFP of OA and cartilage defect patients. The preliminary results have been presented at TERMIS 2020 and the European Society of Tissue Regeneration in Orthopedics and Traumatology (ESTROT) in 2022. Furthermore, the results are planned to be presented at the European Society for Biomaterials (ESB) in 2023. In (pre)clinical setting and future biomedical research, REIMS is used to study the molecular profiles in a variety of tissues and a variety of disease pathologies, allowing it to be used in a wide range of applications. Utilizing this technique on the IPFP of OA and cartilage defect patients can improve the understanding of impaired molecular pathways and pathologies. The data acquired could be used to construct accurate pattern recognition and prediction models to, for example, identify different molecular OA phenotypes, or predict patient outcome after cartilage repair surgery to improve surgical decision making and possibly contribute to the development of (novel) personal treatment strategies.

Not only can REIMS be used on the IPFP, with the right optimization, it can be used on many other tissue types, using less destructive techniques such as laser-assisted REIMS on cartilage[384]. Moreover, REIMS can be applied in the food industry to, for example, study food quality and safety, or for the identification of specific types of species in flora and fauna, or accompanying (resistance to) parasites, bacteria, and fungi. Its application is unlimited, as long as ablation of the tissue and the ionization of molecules is possible with REIMS.