Biomarkers in cartilage repair and osteoarthritis

Summary

Osteoarthritis (OA) is not only affecting the elderly population, but also more and more affecting the younger population, causing not only disability, but also co-existing conditions such as heart disease, diabetes, or mental health problems. Its incidence is expected to increase due to an ageing population, an increase in obesity, and an increase in sport injuries. A variety of intra-articular tissues, including the infrapatellar fat pad (IPFP), contributes to a healthy joint homeostasis. The IPFP is an important inflammatory mediator in the knee joint and has been associated to knee pain after injury, as well as progression of knee OA. A disturbed joint homeostasis due to e.g. injury or an inflammatory event can cause OA in this joint. OA is characterized by a progressive loss of cartilage and has a negative impact on a patient's quality of life. OA is usually diagnosed in late stage of the disease.

At this moment, there is no specific biomarker for early diagnosis of OA, OA progression, or prognosis after cartilage repair surgery. Most studies focus on biomarkers in cartilage, synovium, or synovial fluid. However in this Thesis, the potential of the IPFP as biomarker discovery was addressed. In addition, in Chapter 2, it was discussed that, rather than one specific biomarker, a group of molecules (including lipids, proteins, and metabolites) would provide us with a biomarker panel that gives better insight in OA development and potential therapeutic targets. The use of untargeted mass spectrometry (MS) techniques could contribute to gaining more knowledge on the molecular understanding of OA. Early detection and treatment of OA are of great importance. Life-style changes, as well as joint-preserving surgeries have been shown to slow down, stop, or reverse OA development. As soon as a patient suffers from end-stage OA, the only treatment option available is total knee arthroplasty (TKA). Whereas this treatment has a limited lifespan, postponing OA progression and TKA in patients is of great importance.

The first line of treatment options for OA include exercise and dietary changes, as well as the use of non-steroidal anti-inflammatory drugs (NSAIDs) to treat symptoms. One of these NSAIDs is celecoxib, a selective cyclooxygenase-2 (COX-2) inhibitor. In Chapter 3, the anti-inflammatory and chondroprotective effect of celecoxib were studied in a human cartilage explant culture, as well as an OA animal model by ways of intra-articular single bolus administration. Celecoxib reduced the secretion of pro-inflammatory prostaglandins, as well as proteins, and altered gene expression in human articular cartilage explants. *In vivo*, in a rat OA model, celecoxib acted chondroprotective after a single intra-articular bolus injection.

To investigate the potential of the IPFP in the search for biomarkers for OA, in Chapter 4, a method for the analysis of lipids in the IPFP using matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) was optimized. The biggest challenge lays within the sample preparation. The IPFP is an adipose-like tissue and is therefore prone to melt. Melting tissue during various steps of the sample preparation, including cryosectioning, transportation, or matrix application would cause delocalization of molecules. To prevent this from happening and to keep the IPFPs spatial molecule information, cryosections were made at very low temperatures. Thaw mounting and refreezing were performed at very fast rates and transportation was always performed using silica gel carrier boxes. The main different tissue types within the IPFP explant (adipose tissue, connective tissue, and synovium) could be identified with MALDI-MSI. Subsequently in Chapter 5, this method was applied to visualize the differences in lipid profiles in the IPFP of OA and cartilage defect patients. Different lipid profiles were identified for OA and cartilage defect patients. In addition, the IPFPs intra-tissue heterogeneity was acknowledged, as it might be associated to patient phenotypes. Arachidonic acid-containing, ether-linked phosphatidylethanolamines (PE-O-s) in the connective tissue of the IPFP were suggested specific for OA.

In Chapter 6, we worked towards a point-of-care device for diagnosis of OA, as well as prediction of OA development after a cartilage defect. Rapid evaporative ionization mass spectrometry (REIMS) was used to visualize the lipid profiles of OA, as well as a variety of cartilage defect patients. REIMS was only able to correctly classify cuts made in the IPFP of patients with an age above 35 or below 35 years (65%). Further, as has been shown previously in Chapter 4 and Chapter 5, the IPFPs intra-tissue heterogeneity was of importance. Cuts made in either adipose tissue or connective tissue could be correctly classified with a rate of 90%. Taking into account this intra-tissue heterogeneity while looking at clinical outcome after surgery, the highest correct classifications were acquired with post-operative knee injury and osteoarthritis outcome score (KOOS) for adipose tissue (70%) and connective tissue (73%), as well as for post-operative visual analogue scale (VAS) scores for connective tissue (73%). According to these results, it is not likely that REIMS will be used as method to develop clinical prediction models for cartilage repair surgery. More research and optimization of the technique is necessary to identify the small changes occurring in the IPFP after a cartilage defect.

Proteomic results in Chapter 6 suggest that there is an interaction between the IPFP and cartilage as a variety of cartilage proteins related to cartilage degradation or OA were measured in the IPFP. The IPFP could therefore been seen as promising tissue source for (OA) biomarker discovery. Future studies however, should take into account the IPFP's intra-tissue heterogeneity when drawing conclusions.