Micro-environmental and macro-environmental improvement of joint quality in knee joint pathologies

Impact Paragraph

1. Joint Pathologies: A Major Socioeconomic Burden

Joint pathologies such as knee osteoarthritis (OA) are a major public healthcare problem and cause chronic pain and disability among elderly worldwide[1]. These pathologies have a huge impact on the health-related quality of life[1]. OA is the single most common cause of disability in older adults[2]. Factors such as population ageing and increasing numbers of obese individuals are examples which explain the increasing prevalence of joint pathologies such as OA worldwide[2]. Current management modalities target symptoms such as pain, and depend on the severity of the symptoms and structural disease, but eventually surgical intervention with joint replacement is inevitable[3]. Pharmaceutical treatments are being investigated to reduce the burden of disease in knee OA[3]. In addition, drugs are evaluated in their ability to modify the natural course of knee OA by slowing down or stopping the biological processes underlying tissue damage[3]. Currently, no disease modifying OA drug (DMOAD) is available to prevent OA progression. This leads to a major unmet medical need in the treatment of knee OA[4]. Global health metric data show that the number of disability adjusted life years (DALYs) has increased by 2-fold due to OA during the past 30 years[5]. Furthermore, OA is one of the top leading causes of disability in patients aged from 50-75 years[5]. Given the fact that a large proportion of patients in this age group represent the working population, disability caused by OA causes a great socioeconomic burden. Therefore, optimizing treatment for joint pathologies such as OA are of utmost importance.

Unfortunately, the development of treatments to improve knee joint quality and to modify disease progress in joint pathologies such as OA has not made comparable progress with that of many other chronic non-communicable diseases[4]. This may be related to several challenges that exists in joint preserving treatment strategies. In this dissertation, these challenges are described and a scientific basis is provided that can help to impact these challenges.

2. Improving Cartilage Repair Strategies to Improve Knee Joint Quality

In the first part of this thesis **(Chapter 2 and 3)** we have investigated the chondrocyte phenotype in order to improve cell-based cartilage repair strategies in patients with cartilage defects. We have used chondrocyte culture conditions closely resembling the *in vivo* biophysical environment of the chondrocyte. Our findings published in **Chapter 2 and 3** may stimulate others in the field to take the biophysical environment of the chondrocyte into account when optimizing cellular therapies. Since focal cartilage defects are well-known risk factors for knee OA[6,7], combatting cartilage defects with cellular therapies may lead to preventing or at least delaying in knee OA progression.

Next to an improvement of the chondrocyte phenotype *in vitro*, we also expect that our findings will have implications on the intra-articular joint environment *in vivo*. Knee joint osmolarity is decreased in knee OA[8]. Considering studies showing an *in vitro* anti-inflammatory action of biophysical medium osmolarity on human OA chondrocytes[9,10], we expect that increasing intra-articular joint osmolarity to biophysical levels will act anti-inflammatory on the knee joint *in vivo*. This in turn can lead to improved cellular therapies, but may also delay knee OA progression. Furthermore, a biophysical osmotic environment in the knee joint is expected to protect against iatrogenic injuries to cartilage due to sharp surgical instruments or edges of the arthroscopic sheath during during routine orthopaedic arthroscopic surgery[11]. This is supported by an *in vivo* study showing that articular chondrocytes are less prone to iatrogenic injury due to surgical instruments when the osmotic pressure of the irrigation fluid used during routine orthopaedic surgery is increased[11].

In conclusion, our findings published in **Chapter 2 and 3** provide methods to improve the chondrocyte phenotype and this is expected to facilitate others in optimizing cellular therapies for transition of *in vitro* findings to *in vivo* patient care.

3. Anti-Inflammatory Knee Joint Treatment to Improve Knee Joint Quality

In knee joint pathologies such as knee OA, joint homeostasis becomes disturbed and pathologic changes can be observed in other joint tissues[4]. Our findings in **Chapter 4**, showing secretion of prostanoids by HFP from cartilage defect patients, have important implications. Prostanoids are involved in inflammation and cartilage degradation[12]. The increased presence of prostanoids in the knee joint may be a sign of pre-OA, where regenerative cartilage treatment strategies are less likely to succeed[13]. Therefore, our findings can stimulate the field in future research to correlate prostanoid levels produced by joint tissues to clinical outcomes of cartilage repair surgeries. Moreover, we showed that celecoxib can act anti-inflammatory on the HFP of patients with OA and cartilage defects. Given the fact that an inflamed joint environment is associated with cartilage degradation[13], anti-inflammatory treatment with celecoxib holds potential to create a joint environment more permissive for cartilage repair. Our findings presented in **Chapter 4**, showing secretion of different prostanoid subtypes, can also have important implications as biomarkers for specific processes such as fibrosis in knee OA[14]. Prostanoid subtypes are known to be involved in specific pathophysiological processes of the knee joint: for instance, the prostanoid subtype $PGF_{2\alpha}$ has been shown to be involved in fibrosis in the knee joint[15]. This specific prostanoid subtype thus holds potential as a target to improve knee joint quality in joint pathologies such as knee OA, where fibrosis has been shown to occur[15]. Furthermore, post-operative fibrosis in the knee joint, i.e. arthrofibrosis, has been described to occur after knee surgeries such as anterior cruciate ligament reconstruction surgeries[16]. Therefore, a modulation of $PGF_{2\alpha}$ release in the knee joint holds potential to target post-operative arthrofibrosis.

As earlier mentioned, the development of pharmacological treatments to improve knee joint quality and to modify disease progress in joint pathologies such as OA has not made comparable progress with that of many other chronic non-communicable diseases[4]. This is related to several challenges that exist in developing DMOADs which are inherent

to the disease. Firstly, OA has a slow and long-term natural course, which requires long follow-up periods for DMOADs to detect OA disease modifying effects[1]. Secondly, OA is a heterogenous disease characterized by multiple phenotypes, with a substantial variability in its clinical and radiographic presentation[17]. In **Chapter 4**, we have found a differential inflammatory profile in the HFP from OA patients, which distributed to 2 OA subgroups. It will be interesting to determine whether the different HFP inflammatory states correlate to pain sensation. If this will be the case, then this has a potential impact on the patient specific use of anti-inflammatory treatments for pain relief in knee OA. Considering the fact that also other synovial joints such as for instance the hip, ankle and the elbow joint contain an intra-articular fat pad[18], it is tempting to speculate that these intra-articular fat depots can be a source of pain in patients that have OA in these specific synovial joints. We hypothesize that these intra-articular fat depots may act as internal homeostatic joint regulators and can become a potential source of pain in joint pathologies such as OA.

In **Chapter 4**, we described that celecoxib holds potential as an anti-inflammatory drug that can improve knee joint quality via decreasing secretion of inflammatory mediators by the HFP. In **Chapter 5**, we present a systematic review about the chondroprotective actions of selective COX-2 inhibitors when used systemically or intra-articularly. Our review in **Chapter 5** has provided important insights into the chondroprotective actions of selective COX-2 inhibitors: their chondroprotective action may depend on their administration route. This is a possible explanation why clinical trials, which used the systemic administration route[19,20], failed to show *in vivo* chondroprotective actions with selective COX-2 inhibitors. The intra-articular administration route may be a more effective means, and this can also hold true for other DMOADs. A disadvantage of the intra-articular administration route is the need of frequent injections, which will increase the risk of bacterial knee joint infection[21]. However, it still remains the question whether frequent injections are needed, or whether drug-delivery systems are paramount to incorporate DMOADs in. Moreover, a DMOAD may only be effective in disease modification for a certain OA subtype. Nevertheless, it is likely that drug delivery systems may become important tools to incorporate DMOADs in, considering the long-term natural course of knee OA[3]. Therefore, intra-articular drug delivery system are being developed and are under investigation for treatment in joint pathologies such as

knee OA[22]. Intra-articular drug delivery has a number of advantages over systemic delivery, including increased local bioavailability, reduced systemic exposure, fewer adverse events and reduced cost[22].

In **Chapter 6**, we have evaluated an auto-regulatory drug delivery system and demonstrated its biocompatibility and auto-regulatory release behavior. Considering the pain-killing effects of selective COX-2 inhibitors such as celecoxib which we have investigated in **Chapter 4** and **5**, one has to consider the delicate balance between the pain-killing and structural disease-modifying effects. The auto-regulatory drug delivery system that we have described in **Chapter 6** may provide important advantages compared to a drug delivery system without auto-regulation, especially considering the adverse progression of knee OA that occurred in the clinical trials with for instance anti-NGF[23]. This unwanted adverse progression of OA was possibly due to the fact that a continuously analgesic effect of the anti-NGF caused patients to increase their physical activity and thereby overloading their joints. The FDA has rejected the introduction of anti-NGF into the clinical practice due to the associated risk for joint destruction and rapidly progressive OA. We expect that an auto-regulatory drug release approach will prevent a continuous suppression of pain sensation. This in turn will likely prevent patients to continuously overload their joints, and avoid adverse effects of the treatment. Thus, an auto-regulatory drug release approach provides an attractive balance between drug release and local knee joint trauma. Before implementation of our investigated drug delivery system into the clinical practice, important challenges remain. Firstly, a DMOAD has to be developed that can be bio-actively released from the carrier. As an alternative, an analgesic without adverse effects on knee joint quality may be used. Secondly, sterilization of the system may alter its drug release behavior and few studies pay attention to sterilization of the drug delivery system[21]. Furthermore, contamination with microbial components such as lipopolysaccharides has been described in engineered drug delivery systems and can lead to unwanted toxic side effects[24]. Fourthly, manufacturers of drug delivery systems face regulatory challenges. No uniform definition exists which can classify a certain drug delivery system under a specific category from pharmaceutical science[25]. This prevents the application of a uniform regulation, and certain drug delivery systems may be subject to the European

Union Medical Device Regulation, introducing specific requirements for medicines with an integral device[25]. Cell therapies, coatings and injectables with drug release behavior are upcoming technologies for which new regulations as well as collaborations between public and private partners are paramount.

Patient Selection and Control of Clinical Outcome in Therapies Targeting Knee Joint Quality

In this thesis we have described important strategies to improve knee joint quality. Two important considerations regarding these joint-preserving strategies have to be addressed (Figure 1):

1. Selecting patients that will benefit from the joint preserving intervention
2. Measurement of and control over the clinical outcomes of the joint preserving intervention

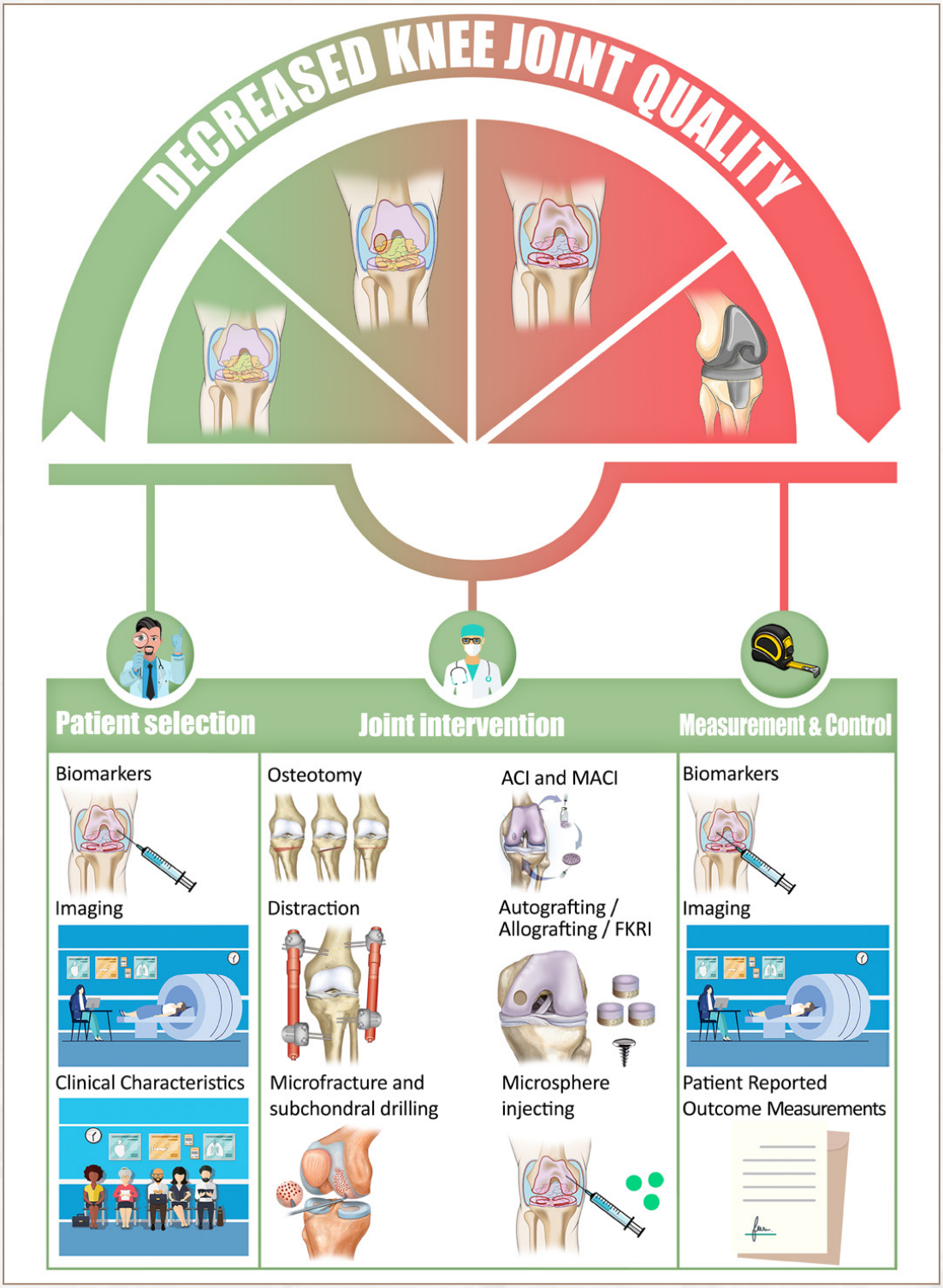
DECREASED KNEE JOINT QUALITY
Patient selection
Joint intervention
Measurement & Control
Biomarkers
Imaging
Clinical Characteristics
Osteotomy
Distraction
Microfracture and subchondral drilling
ACI and MACI
Autografting / Allografting / FKRI
Microsphere injecting
Biomarkers
Imaging
Patient Reported Outcome Measurements

Figure 1: Patient selection, joint intervention and outcome measurement.
Total knee replacement surgery can be performed when knee joint quality is decreased. To postpone a total knee replacement surgery, it is important to select patients that will benefit from a joint preserving intervention. Clinical characteristics, imaging data and synovial fluid biomarkers are attractive candidates to utilize in patient selection. To evaluate the outcomes of joint preserving interventions, synovial fluid biomarkers, imaging data and patient reported outcome measurements may be combined.

The existence of OA phenotypes has to be considered when selecting patients that can potentially benefit from a specific joint preserving intervention[26]. Currently, it is unclear how the various OA phenotypes can be applied to predict outcome of the disease or specific response to joint preserving treatments. Post-traumatic OA, for instance OA developing in patients with cartilage defects, is one example of an OA subtype[6]. Since patients with cartilage defects can develop OA[6], patient selection for joint preserving interventions such as cartilage repair surgery is important to delay or even prevent OA progression in this patient group. Several prognostic factors have already been described for these patients undergoing cartilage repair surgery. Earlier it was demonstrated that factors such as defect location, patient age, the number of treatments and defect age can predict clinical outcomes after cartilage repair[27,28]. For defect age, it was shown that patients with older defects have decreased improvements on clinical outcome after cartilage repair surgery[27]. This is likely to be related to a more disturbed knee joint homeostasis in this patient group, which is expected to inhibit the reparative response in the knee joint. This disturbed knee joint homeostasis can be evaluated by means of certain biomolecular biomarkers. Biomolecular markers may aid in selecting a specific patient group that will benefit from a joint preserving treatment. To date, no single biomarker has been developed to be sufficient for diagnosis or prognosis of joint pathologies such as cartilage defects or knee OA[17]. It is unlikely that in joint pathologies such as knee OA, a single biomarker can be used as a stand-alone measurement that will guide therapeutic strategies. However, these biomolecular markers can be used as an additional tool next to clinical, demographical and radiographical data to guide treatment decisions.

Biomolecular markers can be measured in various body fluids such as blood plasma, urine, serum, lacrimal secretion or synovial fluid. Specifically, the synovial fluid represents a promising body fluid for developing OA biomolecular diagnostics to access the condition of the joint as whole. It is in contact with many of the important intra-articular tissues, and directly represents the status of the knee joint, providing a clear evaluation opportunity of knee joint homeostasis. In **Chapter 7** we have identified the intra-articular tissue of origin for many proteins which are known to be increased in synovial fluid from OA patients compared to non-OA patients. Moreover, we found tissue-specific proteins which were increased in OA synovial fluid compared to non-OA synovial fluid. These proteins are potential candidates as biomarkers in efficacy studies of novel experimental treatments for knee OA, and may be able to report about the joint's health status on the tissue level. Cartilage-specific proteins can be used to aid in clinical decision making for patients with a cartilage defect. Furthermore, these tissue specific proteins hold potential to be used as treatment response biomarkers, aiding in the evaluation of outcomes in cartilage and meniscal repair surgery. In addition, our findings published in **Chapter 7** will fuel the development of novel synovial fluid-based approaches aiming at grading and subtyping knee OA. We expect that grading and subtyping joint pathologies such as knee OA will help to select patients which will benefit from specific treatments such as intra-articular injections with pharmaceuticals or regenerative cartilage repair strategies.

In conclusion, findings published in this thesis hold the potential to limit iatrogenic injuries during routine orthopaedic surgery, and may help to improve cell-based cartilage repair strategies, which can potentially delay knee OA progression. Furthermore, we provided evidence that anti-inflammatory treatment of the inflamed HFP can potentially lead to an improved knee joint quality. Moreover, we reported that the chondroprotective effects of selective COX-2 inhibitors may depend on their administration route. The auto-regulatory drug delivery system that we have described, holds the potential to incorporate analgesics, or DMOADs, to treat pain or improve structural changes in knee OA. Finally, we identified novel potential biomolecular markers that are released from different intra-articular tissues and may be used to assess knee joint quality on a tissue-specific basis. These biomarkers can aid in clinical decision making in joint pathologies such as knee OA and cartilage defects,

by subtyping knee OA and selecting patients that may benefit from the anti-inflammatory treatment with a drug delivery system. For example, the one that we have described in this thesis.

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