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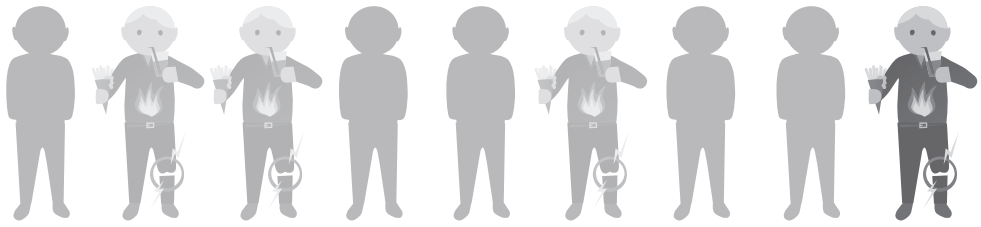
General introduction to osteoarthritis



1.1 Introduction

In 1743 William Hunter stated; From Hippocrates to the present age it is universally allowed that ulcerated cartilage is a troublesome thing and that once destroyed, is not repaired (1). The fact that destroyed cartilage is the main feature of the disease osteoarthritis (OA), makes it that researchers since decades are aiming to find the holy grail how to interfere in the pathogenesis of OA. In general, OA is described as a heterogeneous joint disease characterized by a progressive loss of cartilage.

1.1.1 Facts and figures

Worldwide it is estimated that 9.6% of men and 18% of women aged >60 years have symptomatic OA (2). However, these numbers should be interpreted with caution as the definition of OA is unclear and its onset is difficult to determine. Based on radiographs, OA in the hand joints is most frequently followed by knee and hip (3, 4). In general, OA is more prevalent in Europe and the USA than in other parts of the world (figure 1). OA is a major cause of impaired mobility and it belongs to the top ten of leading causes of burden of disease in high-income countries (5). In the Netherlands, most recent data from General Practices indicate that the prevalence of OA is 29/1000 for males and 50/1000 for females in the adult population (6).

1.1.2 Symptoms

Osteoarthritis is a heterogeneous disorder which is diagnosed based on symptoms, joint pathology or a combination of these two. Symptoms attributable to OA include pain, cracking (crepitus) and stiffness in the affected joints. The presence of osteophytes, joint space narrowing, sclerosis and altered shape of the bone end can be assessed, e.g. by using radiograph, and are in general classified on behalf of the Kellgren-Lawrence grading system (7). The most used diagnostic criteria were developed by the American College of Rheumatology (ACR) (8-10). These criteria include a combination of symptoms (pain) with radiographs.

1.1.3 Risk factors

Several risk factors for the development and progression of OA have been determined. The increase of age is one of the major contributors (figure 1) and is seen in all joints. After the age of 50, the prevalence and incidence of OA in the female gender is significantly greater than in men (11). Other risk factors which are frequently shown to increase OA occurrence are e.g. obesity, sex hormones, ethnicity and race, genetic predisposition and joint trauma (12). The mechanisms by which these risk factors contribute to the development and progression of OA are far from understood.

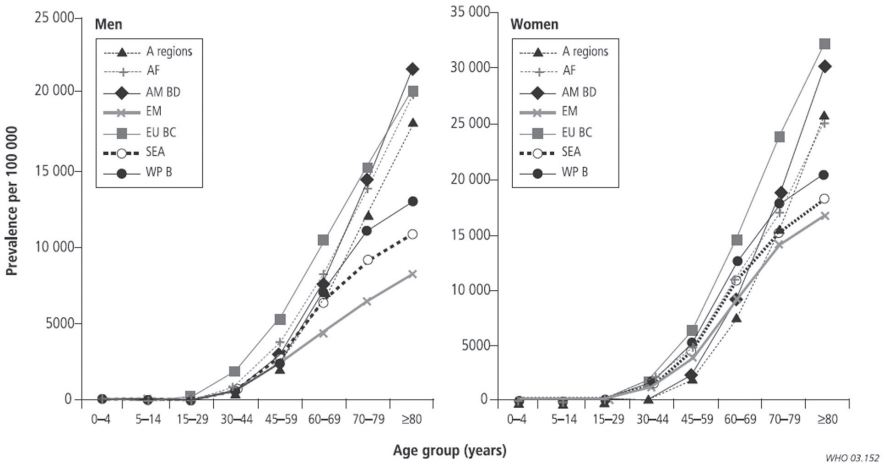


Figure 1. Prevalence of osteoarthritis of the knee, by age group, sex and region in 2000 (World Health Organization). A regions=developed countries in North America, Western Europe, Japan, Australia and New Zealand. AF=countries in sub-Saharan Africa. AM BD=developing countries in the Americas. EM=countries in the Eastern Mediterranean and North African regions. EU BC=developing countries in Europe. SEA=countries in Southeast Asia. WP B=countries in the Western Pacific region (2).

1.1.4 Problems and challenges

Existing therapies are primarily aimed to reduce pain and no cure is available which can interfere in the pathology of OA. Based on different risk factors (and mechanisms involved) and frequently observed differences in disease progression (between patients but also between joints) it becomes increasingly clear that OA is a disease which involves multiple tissues (13).

It is hypothesized that a variety of OA forms may exist that are similar with respect to outcome, however have a different underlying pathophysiological process. This may explain the variable outcomes of clinical trials, biomarker studies and genetic association studies, and therefore the difficulties observed when analyzing the efficacy of novel drugs. Without patient stratification, clinical trials may be 'contaminated' with patients that respond differently to interventions, which results in very large and costly clinical trials and prohibits the process of the development of new disease interfering therapies. To improve prediction of disease outcome, to optimize clinical trial efficiency and to analyze the efficacy of novel drugs, it is crucial to better understand the various mechanisms leading to the clinical outcome of OA.

Currently, OA management is directed toward patients in the latter phase of the disease. Although it is difficult to detect OA in an earlier stage, as radiographs are not sensitive enough, emphasis on early diagnosis and prevention could have more significance. Relevant biomarkers, objective measures that can be derived from body fluids such as blood or urine, are needed to diagnose and forecast OA in an earlier phase of the disease (14). To obtain representative biomarkers it is essential to better understand the role of local and systemic factors, which are involved in the pathogenesis of OA.

1.2 Local alterations; from cartilage to multiple tissue disease

OA has long been considered a wear and tear disease leading to loss of cartilage. During the past decades there have been significant developments in the scientific understanding of OA. These days OA is appreciated as a disease affecting the whole joint which involves complex interactions between several joint tissues (figure 2)(15, 16).

1.2.1 Cartilage

Articular cartilage is a highly specialized avascular connective tissue which provides smooth articulation and bending of the joints during movement. The extracellular matrix of cartilage consists mainly of collagen type II. Collagen type II provides a network in which other constituents, such as proteoglycans and chondrocytes, are

embedded. Collagens and proteoglycans give cartilage the capacity to absorb and distribute loads and to present a low-friction surface (18). Chondrocytes, the only cell type present in cartilage, have very low metabolic activity and are assumed to maintain the extracellular matrix by a low turnover replacement of matrix proteins. During OA development the chondrocytes become “activated”, a process which is characterized by cell proliferation and cluster formation. A disturbed equilibrium develops in which the rate of loss of collagens and proteoglycans goes beyond the rate of the deposition of newly synthesized molecules (19).

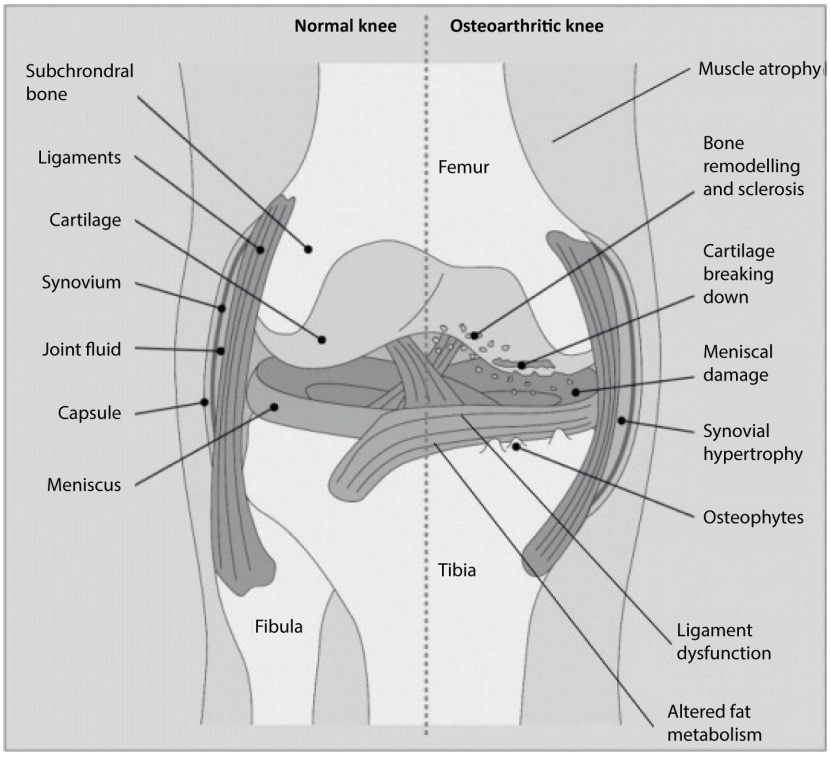


Figure 2. Schematic overview of the normal and OA knee joint depicting the joint tissues affected in osteoarthritis (17)

1.2.2 Synovial tissue

Synovial tissue is in direct contact with articular cartilage and primarily maintains the synovial cavity and synthesizes synovial fluid. Synovial fluid has a lubricating function and facilitates a smooth movement between joints. Under normal conditions, synovial tissue consists of only 1-2 cell layers of macrophage-like (type A) synoviocytes and fibroblast-like (FLS or type B) synoviocytes (20). The inflammation of the synovium (synovitis) has been shown to occur in a number of OA patients and may produce proteases and cytokines that contribute to the disease (21). It has been suggested that activated synovial macrophages play a key role in the processes leading to synovial inflammation. This inflammation may act as a trigger for several symptoms of OA via release of soluble mediators by synovial tissue, thus contributing to the breakdown of cartilage by promoting destruction and impairing the ability of repair (22).

1.2.3 Infrapatellar fat pad

The knee joint contains a special form of adipose tissue named the infrapatellar fat pad (IPFP), which until recently did not receive a lot of attention as a contributor to the OA process. IPFP is located intra-capsularly and extra-synovially in the knee joint, and is in close contact with synovial layers and articular cartilage. Its main role is to facilitate the distribution of synovial fluid and to absorb forces through the knee (23). Considering its location and with regard to OA as a multiple tissue disease it is likely that IPFP is also involved in the pathogenesis of OA (24). Several soluble mediators are locally produced in the knee joint by the IPFP (25, 26). However, the precise role of the IPFP still needs to be elucidated.

1.2.4 Bone, meniscus and ligaments

Subchondral bone, meniscus and ligaments are described to be involved in OA pathogenesis. During OA, bone remodeling takes place as a result of altered joint homeostasis. Hereby, new bone at the joint margins (osteophytes) is formed. In addition, there is evidence available suggesting a link between bone sclerosis and modifications in bone mineralization and the progression of OA. A role for transforming growth factor (TGF)-β, which for example is produced by synoviocytes, is appreciated

in the development of osteophytes (27, 28). Whether subchondral bone sclerosis precedes the onset of OA or is a change that occurs parallel to cartilage degradation is unknown (16, 29). Furthermore, a role for the meniscus and surrounding ligaments is proposed. Meniscal damage occurs in 63% of adults with symptomatic knee OA (30), and it was shown that it leads to a 7.4 times higher chance to develop radiographic knee OA 30 months later (31). These data suggest that bone and meniscus need to be incorporated in the search for new OA targets.

1.2.5 Local Inflammation

Although OA is conventionally not considered as an inflammatory disease, the production of several inflammatory soluble mediators by different tissues in the knee joint suggests that inflammation has a more important role in affecting cartilage homeostasis than originally thought. Until now the most studied cytokines in OA are interleukin (IL)-1β and tumor necrosis factor (TNF)-α. These cytokines are likely to be produced by articular chondrocytes, but synoviocytes may also very well be the source. In clinical trials attempting to block their activity, however, only minimal efficacy was found (32, 33). Both cytokines are mediated through the nuclear factor kappa B (NFκB) cascade, which is a pathway designated to have an important role in OA pathogenesis and a central regulator in catabolic actions in chondrocytes (34). Also, these cytokines are able to initiate other cytokines such as IL-8, IL-6, monocyte chemoattractant protein (MCP)-1 and RANTES (regulated on activation, normal T-cell expressed and secreted), which drive inflammation, inhibit matrix synthesis and promote cellular apoptosis by affecting, for example, aggrecanase (ADAMTS; a disintegrin and metalloproteinase with thrombospondin motifs) and collagenase activities (MMPs; matrix metalloproteinases) (22, 29, 35).

A special class of inflammatory mediators are oxylipins. These mediators are derived from fatty acids and can be found in all tissues in the body and may be interesting targets in relation to OA initiation and perpetuation. A balanced level of oxylipins is essential in maintaining joint homeostasis and alterations have widespread consequences. Prostaglandins are an example of such oxylipins and they have shown to be inhibited by non-steroidal anti-inflammatory drugs (NSAIDs), which leads to reduced OA pain. The precise role of other oxylipins, however, is unknown and merits more extensive research (36-38).

Exploring the role of the different oxylipins and soluble mediators involved and produced by several tissues in the joint, such as the synovial tissue and the IPFP, may lead to potential targets for disease-modifying interventions.

1.3 Systemic alterations; from mechanical to systemic disease

For several decades OA was regarded as a joint disease complicated by mechanical factors and age-related modifications. Limited attention to other factors influencing the disease was given. With the current knowledge, it is widely accepted that OA is more than only a mechanical disease and may be seen as a systemic disorder of multifactorial origin wherein genetic, environmental, hormonal and metabolic factors interact and contribute to OA pathogenesis (39). The metabolic syndrome comprises a profile including a combination of being obese, hypertension, dyslipidemia and impaired glucose tolerance (40). Systemic alterations may be induced by components of the metabolic syndrome. Recently, various studies presented a relation between OA and the prevalence of metabolic syndrome. For example, it was demonstrated that the prevalence of metabolic syndrome is over twofold higher in the OA population (41). In addition, having OA is associated with an over 5 times increased risk of having metabolic syndrome (42). These results have led investigators to contemplate common underlying pathologies in OA and metabolic syndrome related diseases (39, 43, 44).

1.3.1 Obesity

Obesity is an important and strong risk factor for OA and one of the components of the metabolic syndrome (45, 46). Obesity is becoming an increasing problem in the western world. In the Netherlands, 11 % of the adult population is obese (body mass index (BMI) >30) (47). For long, it was thought that the mechanical forces induced by obesity could explain the association between OA and obesity. However, it has been demonstrated that obesity is also associated with hand OA (48). As we do not walk on our hands, this suggests that systemic factors induced by obesity contribute considerably to the initiation and progression of OA (49). Obesity is associated with a mild chronic inflammation and the adipokines secreted by adipocytes and

macrophages within adipose tissue are suggested to be a metabolic link between obesity and OA (50). The relative contribution of these processes in the onset and progression of OA, however, remains unclear.

The association between obesity and the development of OA has been studied in several animal models. In the early 50's it was already discovered that mice receiving a high fat diet (HFD) developed features of OA twice as fast compared to mice fed a normal diet (figure 3) (51). In addition, mice of the STR/ort strain, which are susceptible to develop spontaneous obesity and certain aspects of the human metabolic syndrome, develop OA in a short period of time (52). Furthermore, it has been demonstrated that mice receiving HFD showed more OA cartilage degeneration than those fed a normal diet in a post-traumatic mouse model (53). A very HFD in mice induces OA, but when animals are placed on a wheel-running exercise plan progression of knee OA is inhibited without reduced body fat (54). Furthermore, leptin-deficient (ob/ob) and leptin receptor-deficient (db/db) mice, which have extremely high body weight on a normal diet, are protected from the development of OA (55), which excludes a role for mechanical factors. The fact that adipose tissue of mice can become inflamed under conditions of metabolic stress (e.g. HFD feeding) and secrete a broad spectrum of inflammatory mediators (56, 57), suggests that HFD-fed mice constitute a suitable model to study the role of (metabolic stress-induced) inflammation on the development of OA.

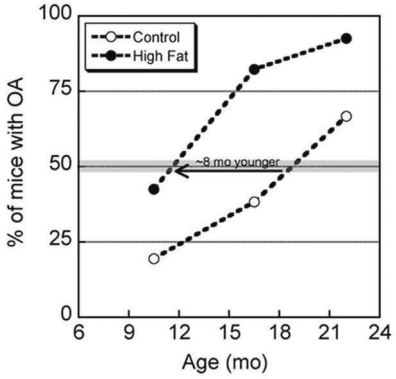


Figure 3. Male C57/Bl6 mice receiving a high fat diet developed osteoarthritis (OA) twice as fast as mice receiving a control diet. The similar slopes indicate that a high fat diet accelerates the onset but not the progression of OA (51).

1.3.2 Cholesterol

Hypercholesterolemia is associated with the metabolic syndrome. Increased levels of cholesterol in the plasma have shown to be associated with generalized OA (58). Furthermore, it has been demonstrated that genes regulating cholesterol efflux have a diminished expression in OA cartilage compared to normal cartilage (59). The underlying mechanism explaining the link between cholesterol and OA features are therefore very interesting. This is supported by the fact that atherosclerosis, which is an atheromatous vascular disease caused by elevated cholesterol intake, is independently associated with OA in women (60, 61). Whether atherosclerosis and OA have common underlying mechanisms or are causative for each other is uncertain.

With respect to animal models, the effects of hypercholesterolemia on OA development is difficult to examine, as wild type mice have a different lipoprotein metabolism compared to humans. The intake of a cholesterol-containing diet by wild type mice leads to elevated plasma cholesterol levels. However, as a result of high levels of anti-atherosclerotic high density lipoprotein (HDL) ('good' cholesterol) and low levels of very low (VL)DL and low (L)DL ('bad' cholesterol) no atherosclerosis develops. Experimental data on investigating the role of cholesterol, and the mechanism behind it in relation to OA development, are therefore scarce and more research needs to be done to elaborate on this association.

1.3.3 Systemic inflammation

The metabolic syndrome induces systemic inflammatory responses. This mechanism of action may also be held responsible for the association of metabolic syndrome with OA, either by a direct effect on articular cartilage, or by the modulation of several tissues in and around the joint. The precise role of inflammation in OA development is, as mentioned earlier, uncertain (chapter 1.2.4) (22, 62). Among the components of the metabolic syndrome, obesity was thought to be key initiator for OA. However, recently published epidemiologic data demonstrated that the metabolic syndrome rather than obesity in itself has the greatest impact on the severity of OA (63). OA donors showed several changed systemic mediators in their serum compared to those obtained from healthy persons (64, 65). Of these

mediators the adipokines, such as leptin, adiponectin, resistin and visfatin, have been extensively studied for their pro- and anti-inflammatory capacities in OA (50). It is found that serum adiponectin, leptin, and resistin concentrations are associated with OA severity and progression and with local synovial tissue inflammation (66-68). The effect of adipokines on the OA process is controversial. Leptin is thought to play a role in TGF-β activation (69) and, as mentioned, extremely obese leptin-impaired mice are protected from the development of OA (55). In addition, leptin induces collagen release from bovine cartilage explants and upregulates MMP-1 and MMP-13 in bovine chondrocytes (70). It has been demonstrated that adiponectin alters the balance by downregulating MMPs and upregulating TIMPs. However, it is also believed that it may act protective against OA by reducing the production of pro-inflammatory cytokines (71). Adiponectin-treated chondrocytes produce IL-6, MMP-3 and MMP-9 and this adipokine induces MMP-1 and IL-6 production in synovial fibroblasts (72, 73). A relatively newly discovered adipokine, visfatin, has been suggested as a promising target for treating OA. Visfatin synthesis is increased by IL-1β treatment in an *in vitro* culture of human chondrocytes. Furthermore, visfatin increases the synthesis and release of MMP-3, MMP-13, ADAMTS-4, and ADAMTS-5 and decreases aggrecan production in chondrocytes, suggesting a pro-inflammatory function in cartilage (74, 75). With regard to cholesterol, it is postulated that oxidized low density lipoprotein triggers pro-inflammatory cytokine production (76). It should be noted that these mediators are not solely originating from the blood and that synovial tissue (21) and IPFP (24) secrete a number of these inflammatory factors as well, which may contribute to OA. Whether targeting the inflammatory response induced by components of the metabolic syndrome, such as adipokines, will reduce OA development is unknown. The integration of knowledge of the metabolic syndrome and related diseases in OA research provides new leads to tackle the underlying mechanisms responsible for OA pathogenesis.

1.4 Outline of the thesis

The aim of this thesis is to provide insight in the role of multiple local and systemic factors contributing to the pathogenesis of OA. With the current knowledge, more advanced technologies, and novel animal models, we should be able to shed a different light on the tissues involved in OA pathogenesis. Hopefully, this will ultimately lead to the identification of mechanisms that provide targets for disease-modifying therapies and novel biomarkers to detect OA in an earlier phase. With respect to the future, we try to contribute to a rationale for a better stratification of OA patients and consequently to more personalized medicine.

The first three chapters include a description of local factors that possibly contribute to OA development. By investigating the secretion of several mediators by synovial tissue, IPFP and synovial fluid, a profile of OA and normal (non-OA) donors is provided.

In **Chapter 2** we aimed to get insight in the role of synovial tissue. We hypothesized that synovial tissue derived from end-stage OA patients is more inflamed than that from normal donors (21). The synovial tissue of OA donors may therefore secrete a wide array of factors that can alter cartilage degradation, which is not the case for synovial tissues derived from normal donors. To test this hypothesis, we used a multiplex approach (an advanced technology including multiplex bead-based immunoassays (77)). In addition, the ability of OA and normal synovial tissue to initiate degeneration of healthy cartilage was assessed by culturing synovial tissues and healthy cartilage explants in a complex *in vitro* co-culture transwell system.

The purpose of **Chapter 3** was to explore the role of IPFP in OA. Oxylipins, important signaling molecules involved in the modulation of inflammatory responses (78, 79), were detected using a mass spectrometry method to get an overview of what is secreted by the IPFP. Furthermore, differences in IPFP secretion between OA and normal donors were evaluated.

We profiled synovial fluid samples as representatives of the secretion of soluble mediators by different tissues involved in the joint (**Chapter 4).** Again we used a multiplex approach to get a wide and comprehensive overview of OA and normal donors. Data were further explored using multi-variate statistics to identify clusters of interrelated mediators.

Besides the involvement of local factors we examined the development of OA with regard to alterations on a systemic level. Considering the hypothesis that obesity might lead to metabolic stress, which possibly contributes to the development of OA (62), we used a HFD-induced OA mouse model which is described in **Chapter 5**. More specifically, we used the human C-reactive protein (hCRP) transgenic mice (80). CRP is an acute phase protein and an established marker for systemic inflammation in humans (81). Consequently, we were able to monitor the inflammatory state of the animals, using a human marker, during the development of OA. To perceive whether medicines applied for other targets in the metabolic syndrome were able to counteract OA-related changes, we included a prophylactic intervention with statins (cholesterol-lowering drug) and a peroxisome proliferator activated receptor (PPAR) agonist (anti-diabetic drug).

In **Chapter 6** we emphasized on lipid and systemic alterations induced by elevated cholesterol intake instead of HFD. We investigated whether a cholesterol-containing diet and, consequently, the development of atherosclerosis was sufficient to induce OA in APOE*3 Leiden.CETP transgenic mice. This is a well-established model for hyperlipidemia and atherosclerosis and resembles the human lipoprotein metabolism in contrary to wild type mice (82-84). Furthermore, we included cholesterol-lowering interventions, with a statin and ezetimibe, in groups receiving a high cholesterol diet, to assess their effects on OA development. Both drugs diminish cholesterol levels to a same extent but have a different mode of action, which allowed us to investigate possible additional effects of statin beyond its cholesterol-lowering capacities.

To elaborate more on the outcomes of chapters 5 and 6, in **Chapter 7** we extrapolated the HFD-induced OA mouse model to the APOE*3 Leiden.CETP mouse. We included an early ('prophylactic') and late ('therapeutic') intervention with a statin. Furthermore, we included a fenofibrate (cholesterol-lowering drug) intervention, since another study indicated that this type of drug has beneficial effects on OA development. To gain more insight into the mechanisms behind the HFD-induced OA model, the effect of a caspase-1 inhibitor, responsible for the conversion of pro-IL-1 to IL-1 (one of the key cytokines believed to be involved in OA), was studied. In addition, a group that started on HFD, but returned to control chow diet halfway the study, was included to evaluate the effect of a diet switch on OA development.

In the final chapters, **Chapter 8** and **Chapter 9** (in Dutch), a comprehensive overview of the performed work is given and discussed.

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