Effects of Dopamine Receptor Activation on Synovial Fibroblasts Obtained from Rheumatoid Arthritis and Osteoarthritis Patients

Inauguraldissertation zur Erlangung des Grades eines Doktors der Medizin des Fachbereichs Medizin der Justus-Liebig-Universität Gießen

vorgelegt von Lina van Nie
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Gießen (2024)

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Contents

Chapter 1

Introduction

Chronic joint pain in the hands and feet together with morning stiffness are characteristic symptoms of rheumatoid arthritis (RA). Oftentimes, patients are not able to dress themselves or participate in daily life, let alone to pursue their profession.[1]
Depression, social isolation, and unemployment frequently lead to additional comorbidities and pose a burden for social security systems. To avoid this situation, it is crucial to uncover the intrinsic and extrinsic factors contributing to the development of RA and identify novel therapeutic targets.

RA is a chronic autoinflammatory disease that not only causes pain but also results in severe destruction of the affected joints, leading to malposition and loss of function. Moreover, RA patients have a higher risk for comorbidities such as myocardial infarction and insulin resistance.[2] Even though there have been recent advancements in the diagnosis and treatment of this disease, it remains incurable and often requires lifelong medication.

During recent years, significant research has been carried out to discover more effective therapeutic options for patients with RA. This has led to the discovery of a strong connection between the immune system and nervous system in this context. For example, neurons have been found to express Toll-like and cytokine receptors,[3,4] while immune cells have been found to express receptors for neurotransmitters such as acetylcholine and dopamine.[5–7] It is notable that receptors for both neuronal and immune signaling have been found on each other's cell types.
Based on recent studies, there is a growing understanding of how the immune and nervous system interact, particularly in chronic inflammatory diseases including rheumatoid arthritis. Dopamine has been identified as having a significant function in inflammatory processes, even beyond the boundaries of the

central nervous system. This is the reason why dopamine is now considered not only a neurotransmitter, but rather a "neuroimmunotransmitter",[7] emphasizing the important role of this catecholamine.

There is also clinical evidence of a dopaminergic effect on RA. For instance, individuals who experience schizophrenia have a lower likelihood of developing RA.[8,9] Additionally, RA patients frequently develop a restless leg syndrome (RLS), which is caused by impaired dopamine function.[10] As RA patients require enhanced therapeutic options, we performed a study to investigate the role of dopamine in the pathogenesis of RA. A significant part of the results have already been published in Scientific Reports in July 2020.[11]

1.1 Rheumatoid Arthritis

Rheumatoid arthritis is a chronic joint inflammation that initially targets the small joints of the hands and feet in a symmetrical manner.[12] However, since several extraarticular symptoms are also associated with this disease, it is more appropriate to consider it a systemic disease with both articular and extraarticular symptoms.[13] The disease affects about 0.5% to 1% of the world's population,[12,14–16] with women being affected twice as often as men.[17]

As with many other autoimmune diseases, a positive family history is associated with a significantly increased risk of developing RA in the future, suggesting a genetic role in the pathogenesis of the disease.[14] In this regard, the HLA DRB1 allele appears to be the most important one.[18–20] On the other hand, RA is also more common in patients of low socioeconomic status and smokers.[13] Twin studies suggest that non-genetic factors, such as the microbiome and epigenetics, play a role in the development of RA, thus implicating environmental factors. However, despite numerous studies exploring various theories of RA pathogenesis, our understanding of the underlying mechanisms and triggers remains incomplete at present.

1.1.1 Symptoms and diagnosis

Symptoms of RA can vary widely from patient to patient. This makes it difficult to distinguish from other diseases, such as osteoarthritis. As a result, many RA patients suffer for years before getting diagnosed and receiving appropriate treatment. Also, the early symptoms of RA are often non-specific: swollen joints with morning stiffness combined with general symptoms such as fatigue, subfebrile temperatures, weight loss, and anemia. This complex situation underlines the multisystemic character of RA.

However, the most dominant symptom of RA is severe pain in the metacarpophalangeal (MCP), carpal and proximal interphalangeal (PIP) joints, combined with morning stiffness that lasts more than an hour. These symptoms are often accompanied by tenosynovitis, which further impairs mobility of the joints. If left untreated, RA can quickly cause joint destruction with subsequent immobilization and malposition. Typical hand malpositions in advanced stages include the swan neck deformity and boutonniere deformity, often accompanied by ulnar deviation in the MCP joints (Fig. 1.1).

Swan neck deformity is characterized by hyperextension in the PIP joint and flexion in the distal interphalangeal joint (DIP), while boutonniere deformity refers to the opposite case involving flexion in the PIP joint and hyperextension in the DIP joint. In severe cases, this condition can result in fixed subluxation of the joints, significantly limiting the functionality of the hands. When the feet are affected, the metatarsophalangeal joints are typically involved, which can also lead to lateral deviation of the toes, similar to the ulnar deviation in the hands. As the disease progresses, an increasing number of joints become affected over time, with symptoms spreading to larger joints such as the knees, shoulders, and hips. While joint pain is the most dominant symptom, several extraarticular symptoms may also be observed. Unspecific symptoms such as weight loss, subfebrile temperatures and fatigue often accompany the course of disease. The inflammatory processes are not limited to the joints and may also affect the lung, pleura and pericardium. Consequently, pericardial and pleural effusion, as well as interstitial lung fibrosis, can result in respiratory distress and facilitate also pneumonia. This underscores the systemic and "syndrome-like" nature of RA.

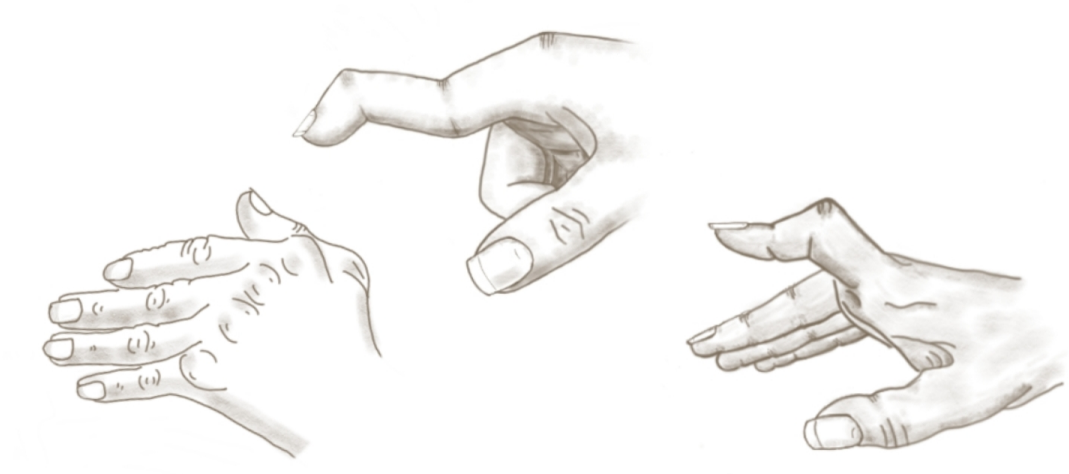


Figure 1.1
Deformities of the hands in RA. Chronic inflammation in the joints of the hands and feet leads to typical deformities over time. Chronic inflammation and subsequent destruction of the MCP joints, in particular, result in progressive ulnar deviation of the fingers (left). Furthermore, inflammation of other joints of the hands and tendinopathies leads to typical finger deformities with loss of function over time. The two most frequently observed deformities in this context are the boutonniere deformity, characterized by hyperextension in the PIP joint and flexion in the DIP joint (middle), and the swan neck deformity, which involves flexion in the PIP joint and hyperextension in the DIP joint (right).
(Figure: Own design)

Approximately one third of the patients show rheumatic nodules that can lead to further impaired joint mobility. Also, patients with RA have a notably increased risk of developing cardiovascular diseases like myocardial infarction and stroke,[2] as well as metabolic disorders like dyslipidemia and diabetes.[2]

Interestingly, patients with rheumatoid arthritis (RA), who have a low body mass index (BMI), face a significantly elevated risk of experiencing and dying from cardiovascular comorbidities compared to patients with normal or higher BMI levels.[2] In fact, RA patients have a 50% increased risk of dying from cardiovascular disease compared to the general population,[21] which is why the European League Against Rheumatism (EULAR) recommends that RA patients be regularly evaluated for cardiovascular risk factors and symptoms.[22] However, modern RA therapy can significantly decrease this risk.[23,24]

RA patients are not only at higher risk for cardiovascular diseases: as in many other chronic inflammatory diseases, the prevalence of depression is much higher in RA patients than in the rest of the population.[25] As RA patients are frequently under immunosuppressive medication, they are also particularly vulnerable to infections. Additional prophylaxis like vaccinations can reduce the risk of severe infections. Together with frequent reevaluation and - if neccessary - adaption of the current medication can reduce the risk of infections. But even without glucocorticoid and DMARD-intake (disease modifying antirheumatic drugs), RA patients are more susceptible to infections.[26,27] This further correlates with disease activity,[27] emphasizing the importance of proper and early treatment.

Other comorbidities often remain silent for several years or even decades, like osteoporosis. Loss of bone density can be observed in the early stages of the disease,[28] although frequently it only becomes noticeable when bones fracture. Even low-energy traumas can result in severe fractures, leading to impaired mobility, increased pain, surgery, and hospitalization. Regular bone density screening is therefore strongly recommended for patients with RA, especially female patients who have been through menopause.
Bone loss results from a combination of various factors. High levels of inflammatory cytokines itself promote bone resorption.[29] In addition, inflamed joints cause pain and, as a result, reduce physical activity, which leads to further bone resorption. Lastly, patients are treated with glucocorticoids to control inflammation and enable physical activity. In fact, glucocorticoid-induced osteoporosis (GIOP) is a common side effect of prolonged use of glucocorticoids,[30] which is why prevention and treatment of osteoporosis is a crucial aspect of treating RA.

When surgery is necessary, several studies show that RA patients face a higher perioperative risk of infection, independently from previous immunosuppressant treatment .[31,32] Nevertheless, treatment with glucocorticoids[33] and DMARDs further increase this risk.[34–36] Additionally, long-term and high-dose glucocorticoid use can lead to dermal and epidermal atrophy.[37] This not only makes them more susceptible to impaired wound healing after surgery, but also to pressure ulcers when mobility is still impaired the first days after surgery. This can be a focus for further infection and complications.

Although rheumatoid arthritis tends to get worse over time for most patients, the course of the disease varies enormously from one patient to the next. While

certain patients experience significant joint destruction that rapidly progresses despite intensive therapy, others require only low doses of treatment.

Over the years, several diagnostic and prognostic factors affecting the course of the disease have been identified.
Rheumatoid factor (RF), an autoantibody against the Fc fragment of IgG, is a significant factor associated with a more severe course of disease. Other negative prognostic factors include early joint erosion and the presence of extra-articular symptoms.[38] Furthermore, the onset of rheumatoid arthritis after the age of 60, also known as late onset RA (LORA), is often associated with a more severe disease course, although studies have reported conflicting results.[39–43] The presence of RF in blood is further associated with increased mortality.[44,45]

However, the presence of RF in blood is not unique to RA and can also occur in other diseases such as hepatitis C or even in healthy individuals who never develop arthritis.[46] About 80% of patients with rheumatoid arthritis (RA) are seropositive, meaning that RF can be detected in the patient's blood,[47] and some initially RF-negative ("seronegative") patients turn RF-positive ("seropositive") over time.
Anti-citrullinated protein antibodies (ACPA) are also frequently found in the blood of RA patients. Compared to RF, ACPAs are more specific to RA[48] and can be found in 80% of RA patients. The combination of ACPA and RF further increases the sensitivity for diagnosis of RA.[49]

ACPA levels are often elevated at the onset of the disease, making it an important diagnostic tool. Interestingly, elevated levels of RF and especially ACPA can be detected in the blood already several years prior to the onset of disease.[50] These levels decrease with adequate treatment.[51] Moreover, B-cell depletion by rituximab has been shown to successfully reduce RF-levels, and at the same time improve clinical symptoms.[52]

In RA patients, in addition to the aforementioned autoantibodies, elevated levels of serologic markers of inflammation, including C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), associated with anemia of chronic inflammation, are commonly observed.[53,54]
Joint involvement can sometimes be detected several years before the onset of symptoms by using imaging techniques such as X-ray, MRI, or ultrasound. The ultrasound scan is a popular technique for detecting synovial inflammation and hyperproliferation, especially in the small joints of the hands and feet, because

of its lack of radiation and ease of availability. Because clinical appearance and radiographic or ultrasound scan findings do not always align, diagnosis of RA requires a combination of laboratory values, radiologic and clinical examination, as well as a thorough assessment of medical history.

In 2010, the American College of Rheumatology (ACR) published classification criteria to further improve the early diagnosis of RA. It is noteworthy that the 2010 ACR criteria do not require imaging techniques such as X-ray to diagnose RA. This underscores the fact that clinical presentation and radiological findings may not correlate, especially in the early stages of RA.[55,56] The 2010 ACR/EULAR classification criteria for rheumatoid arthritis consist of four groups: joint involvement, serology (RF, ACPA), acute phase reactants (CRP, ESR), and duration of symptoms. An analysis of the scores in each category is performed, and if a total of 6 or more points is reached, the diagnosis of RA can be made (Table 1.1).
Patients who do not achieve the required score, however, should remain under frequent observation, as the score may increase over time.[54] When evaluating joint involvement in clinical examination, the ACR considers included joints that are swollen or tender as involved. However, the distal interphalangeal joints, the first carpometacarpal joints, and first metatarsophalangeal joints are not included in the scoring system. The ACR classification criteria are designed to test the most dominant symptoms of RA, helping not only to diagnose RA at an early stage, but in consequence also to prevent the complications and comorbidities mentioned above.

Table 1.1
The 2010 ACR/EULAR classification criteria for rheumatoid arthritis[54]

Joint Involvement	**Score**
1 large joint	0
2-10 large joints	1
1-3 small joints (with or without involvement of large joints)	2
4-10 small joints (with or without involvement of large joints)	3
>10 joints (at least 1 small joint)	5
Serology (at least 1 test result is needed for classification)	
RF and ACPA negative	0
low positive RF or low positive ACPA	2
high-positive RF or high-positive ACPA	3
Acute-phase reactants (at least 1 test result is needed for classification)	
normal CRP and normal ESR	0
abnormal CRP or abnormal ASR	1
Duration	
<6 weeks	0
$\geqq$ 6 weeks	1

By achieving six or more points on the scoring system, the diagnosis of RA can be confirmed. "Large joints" include shoulders, elbows, hips, knees, and ankles, while "small joints" refer to the metacarpophalangeal joints, proximal interphalangeal joints, second through fifth metatarsophalangeal joints, thumb interphalangeal joints, and wrists. ESR = Erythrocyte sedimentation rate. CRP = C-reactive protein.

1.1.2 Pathology

The healthy joint

A joint is the mechanic link between two bones, allowing for the movement and flexibility of the skeleton. There are different types joints, but the subgroup of "true" joints is the most relevant and predominantly affected group in RA (i.e. diarthrodial joints). The ends of two or more bones that form a "true" joint are covered with hyaline cartilage. This allows pressure to be absorbed and evenly distributed over the entire joint surface (Fig. 1.2).

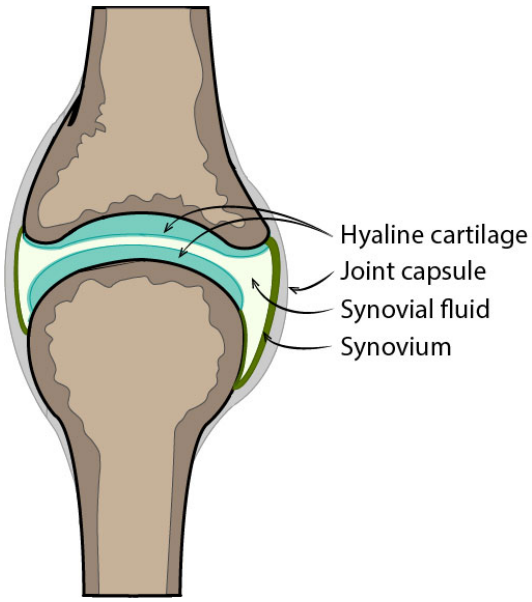


Figure 1.2
Healthy joint with intact hyaline cartilage surface for equal pressure distribution within the joint. The synovial fluid, produced by the inner layer of the joint capsule (synovium), provides nutrition for the avascular hyaline cartilage.
(Source: Own illustration)

Joint cartilage lacks blood vessel supply (bradytrophic tissue) and nutrition is therefore only provided by the synovial fluid, a viscous, clear fluid that fills the space between the two cartilage surfaces. The composition of the synovial fluid is critical to enable normal, pain-free joint movement and weight-bearing. It is consistently produced in the synovial tissue and contains, alongside components for nutritional support of the cartilage, glycosaminoglycans, particularly hyaluronic acids. These hyaluronic acids are negatively ionized and have a high water-binding capacity, which improves the shock-absorbing properties of the cartilage, e.g. during running. Interestingly, the capability for the synovium to produce hyaluronan is significantly impaired under inflamed conditions.
When damage occurs in the cartilage, healing is impaired as the lack of blood vessels makes it almost impossible for stem cells and necessary cytokines to reach the site of injury, significantly reducing the cartilage's ability to heal. Additionally, chondrocytes are highly sensitive to their surroundings and lose their ability to produce extracellular matrix such as collagen Type II and pro-

teoglycans.[57] This clarifies how cartilage damage in both RA and OA results in chronic pain and diminished functionality in the long run, as intact cartilage is essential for a well-functioning joint.

The joint is surrounded by the joint capsule, which separates it from surrounding structures. The joint capsule can be divided into two parts: the outer layer, known as the articular capsule, and the inner layer, called the synovial membrane. The articular capsule surrounds the entire joint, being made of tough fibrous material that is anchored to the adjacent bones. Additionally, ligaments are often integrated into the outer capsule.[58]
The synovial membrane lines the joint cavity and is in direct contact with the synovial fluid. The synoviocytes in the synovial membrane can also be divided into two subtypes: Synoviocytes type A and type B.[59,60]
While type A synoviocytes, or macrophage-like synoviocytes, originate from blood monocytes and are capable of phagocytosis, type B synoviocytes, or fibroblast-like synoviocytes, are resident fibroblasts.[61,62] Type B synoviocytes are the predominant cell type in healthy synovium and produce synovial fluid, which is critical for nourishing cartilage.[61]
Within the synovial membrane, two layers can be distinguished, namely the lining layer and the sublining layer. The lining layer is the innermost layer of the joint capsule and consists of approximately 1-3 layers of tightly packed synovial cells type A and B, with the majority of cells being synovial cells type B.[61]

Under inflamed conditions like in RA, this layer can grow up to 10-15 layers by infiltration of CD68+ macrophages[61] and proliferation of fibroblasts. The sublining layer has a significantly lower cell density compared to the lining layer. It is mainly composed of extracellular matrix such as collagen type I, blood and lymph vessels, and sympathetic nerve fibers. The cell density within the sublining layer is significantly lower in comparison to the lining layer. Additionally, it contains a small number of lymphocytes, macrophages, and fibroblasts.[61]
While the healthy sublining layer has only a few immune cells, the number of immune cells increases massively in inflammation.

The joint in RA

In RA, the joint's physiological structure deteriorates over time due to chronic inflammation that perpetuates itself. When closely examining the joint affected by rheumatoid arthritis, the synovium can be observed as the primary site of inflammation. Taken together, immune cells infiltrate the synovium, releasing pro-inflammatory cytokines that further enhance inflammation, and synovial fibroblasts type B convert into aggressive, proliferating, matrix-degrading cells. This persistent inflammation leads to the loss of the joint's anatomical structure over time.

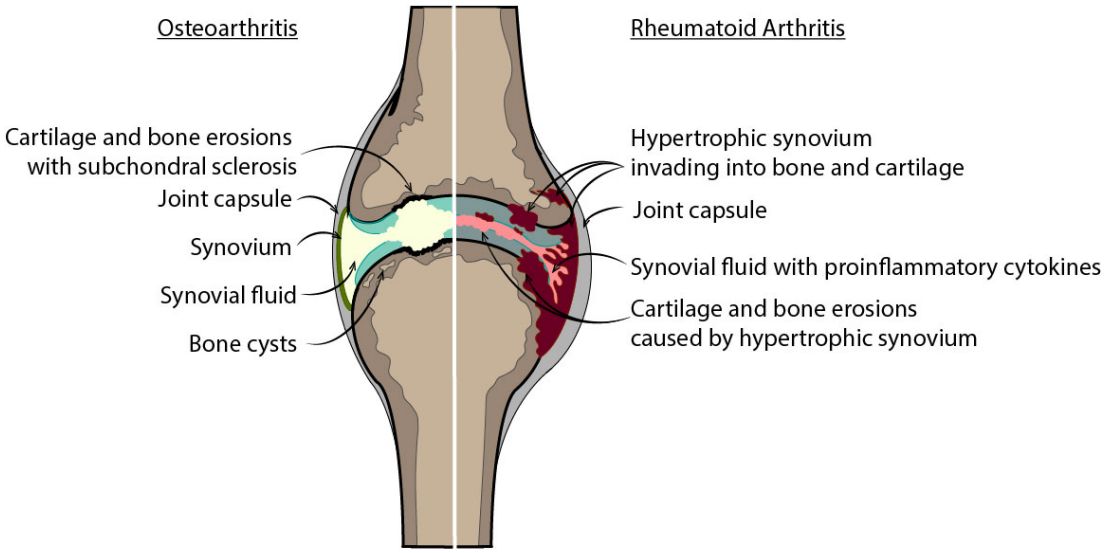


Figure 1.3
In osteoarthritis, the joint is damaged over time due to increased stress from various causes. In the later stages, this results in the erosion of cartilage and bone, accompanied by the formation of bone cysts. Unlike rheumatoid arthritis, there is no primary inflammation causing the joint damage. In RA, constant proinflammatory stimuli lead to hypertrophy of the synovial membrane. This "pannus tissue" aggressively invades the adjacent cartilage and bone structures. Proinflammatory cytokines are secreted from the synovium into the synovial fluid, worsening inflammation and joint destruction.
(Source: Own illustration)

When analyzing the synovium of patients with RA under the microscope, the hypertrophic lining layer is the dominant feature. This layer thickens due to hyperproliferation of synovial fibroblasts (RASF) and macrophages, growing up to 15 layers and forming what is known as the "pannus" (Fig. 1.4 and Fig. 1.5). Notably, RASF in the lining layer exhibit significant differences in morphology and protein expression compared to those found in the sublining layer.[63] Furthermore, this pannus invades the joint cartilage, ultimately leading to its destruction.(Fig. 1.3 and 1.6) The hypertrophic synovium can also mechanically interfere with joint motion because of the limited space in the joint cavity.

This aggressive invasion of RASF into adjacent joint structures are key aspects of RA pathogenesis and joint destruction[64] (Fig. 1.6). The migration potential of RASF is further promoted by the hypoxic environment in the inflamed joint cavity.

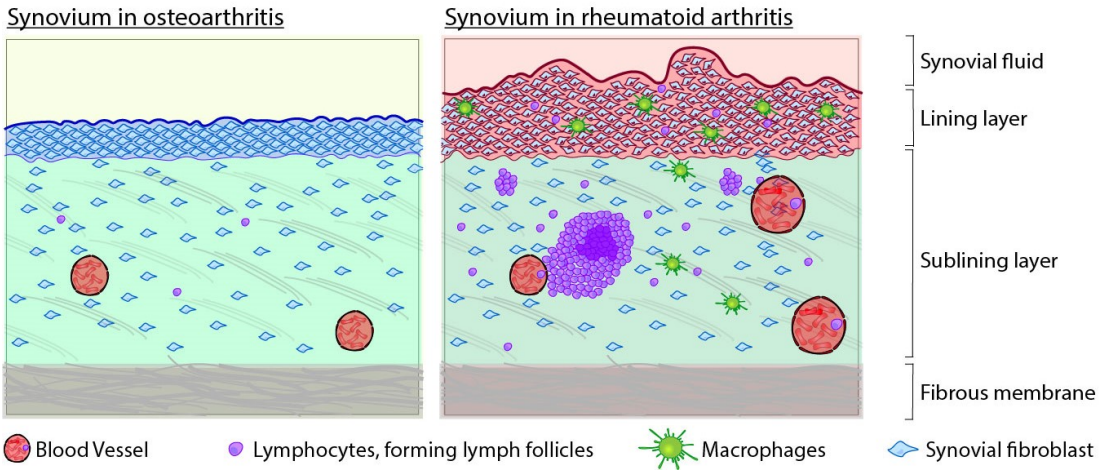


Figure 1.4
Comparison of Synovium in OA (left) and RA (right). In healthy joints and also in OA, the lining layer only counts 1-3 layers. Cellular density in the sublining layer is lower compared to RA synovium and only few blood vessels spread into the synovium. The sublining layer has a lower cellular density when compared to the synovium of RA, and only a few blood vessels extend into the synovium. In contrast, the lining layer in RA has up to 15 layers and forms synovial villi which can mechanically impede joint movement. Furthermore, immune cells such as macrophages and lymphocytes infiltrate especially the sublining layer and form inflammatory infiltrates. This highly inflamed environment also promotes the growth of blood vessels into the synovium. . (Source: Own illustration)

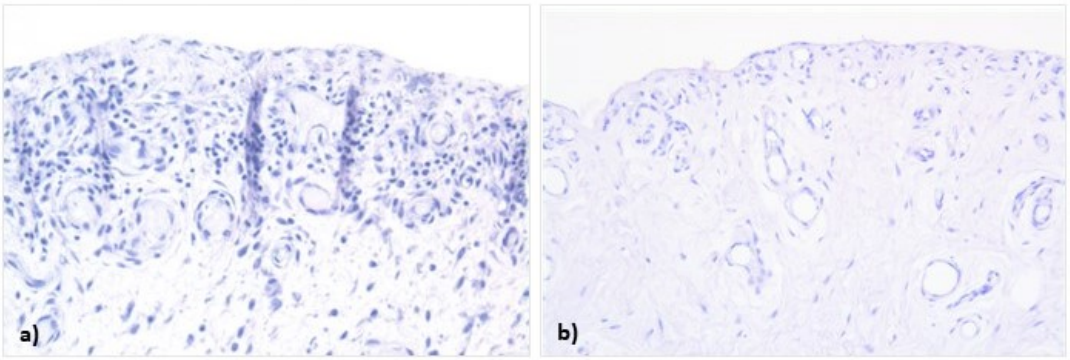


Figure 1.5
Hematoxylin-Eosin-stained paraffin-embedded sections of RA (left) and OA (right) synovium. The lining layer is more prominent in RA synovium than in OA synovium. Moreover, the cell density in RA synovium is much higher than in OA synovium. Both synovial stainings show blood vessels extending into the synovium; typically, there are more blood vessels in RA synovium than in OA synovium. Especially in RA synovium a much higher cellular density around blood vessels can be observed. (Source: Own histologic samples)

RASF exhibit impaired regulatory mechanisms including anchorage-independent growth with loss of contact inhibition[65] and reduced apoptosis rates.[66] At the site of inflammation, proteins from the proto-oncogene family are highly expressed and also epigenetic alterations have been observed.[67,68]
In addition to RASF, the synovium contains various types and subsets of immune cells, some of which are organized into lymphoid follicles. The hypoxic and acidic environment in the synovium also promotes blood vessels to spread into the synovium which enables immune cells reach the site of inflammation easier. Furthermore, it has been demonstrated that RASF possess immunomodulatory effects, including interaction with endothelial cells to influence leukocyte recruitment in rheumatoid arthritis.[69,70]
Compared to OA, the concentration of immune cells in the synovium is highly elevated. The combination of proinflammatory cytokines including TNFα, IL1β and IL6 as key cytokines produced by T-cells and macrophages further promotes and maintains inflammation.[68] TNFα plays a critical role in RA as it promotes immune cell attraction, enhances proinflammatory cytokine production, and contributes significantly to pain signaling.[71] The inhibition of TNFα and its pathway is therefore successfully used in RA therapy.

Besides proinflammatory cytokines, both RASF and local immune cells also produce anti-inflammatory cytokines like IL10, IL13 and TGFβ. Unfortunately, the massive production of proinflammatory cytokines like TNFα neutralises and outweighes the effects of the anti-inflammatory cytokines.

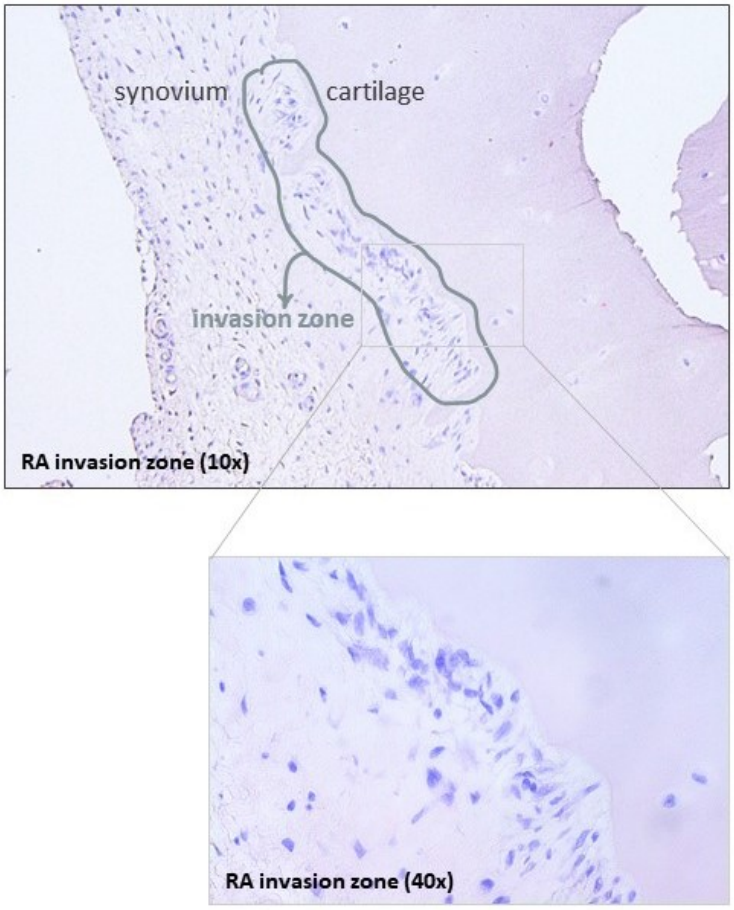


Figure 1.6
HE-staining of paraffine-embedded invasion zone in RA, where aggressive RASF invade into the joint cartilage. RASF invade into cartilage which leads to joint destruction over time. This "aggressive phenotype" of RASF is unique for RA and differentiates it from OA.
(Source: Own histologic samples)

When removed from the inflamed environment, RASF continue to produce proinflammatory cytokines, such as IL6 and matrix metalloproteinases[12,72] This phenomenon was observed both in cell culture and in the SCID (SCID = severe combined immunodeficiency) mouse model. For the latter, RASF and normal human cartilage were translocated into SCID mice, where RASF continued to both invade the cartilage and produce proinflammatory cytokines even in the absence of other immune cells.
Furthermore, Lefèvre et al. showed in 2009, that RASF are able to spread to unaffected joint areas or joints in the SCID mouse model.[73] It is therefore apparent that RASF not only respond to activated immune cells, but also intrinsically transform into aggressive, matrix-destroying cells which is why RASF are commonly referred to as "aggressive phenotype synovial fibroblasts." This tumor-like characteristic of "metastasizing" to other joints is just one of several

tumor cell resembling characteristics of RASF,[74] such the above mentioned increased expression of proto-oncogenes.

Under healthy physiological circumstances, synovial fibroblasts (SF) are responsible for maintaining extracellular matrix homeostasis in the synovium. They synthesize proteins, including collagen type II, for extracellular matrix formation. In addition, SF release enzymes to degrade the matrix, which is for example crucial for wound healing.
However, in RA, this balance between matrix construction and destruction is impaired at the expense of matrix construction.[68] Matrix degradation is mediated by matrix metalloproteinases (MMPs) such as MMP3, which are released into the synovial fluid by the RASF .[63] Moreover, MMP expression, such as MMP13, in the synovium also correlates with elevated serologic markers of inflammation.[63]

Immune cells and synovial fibroblasts are both involved and responsible for the ongoing inflammation and destruction in RA. But what causes the inflammation in the first place? T cells are believed to play a central role in the onset and progression of RA. They are in focus, because the presence of special HLA-DRB1 alleles, coding for MHC II receptor, is strongly associated with the development of RA[75] (the “shared-epitope-theory”) and also with a more severe course of disease.
Disease-associated HLA-DRB1 alleles share a certain amino acid sequence called the shared epitope (SE). Several HLA DRB1 alleles have been linked with an increased risk of developing RA. For example, the *0101-SE-allele is the most common allele in Greece and is associated with a milder disease course compared to western Europe. Some ethnic groups, such as the Pima Native American tribe, exhibit a significant 7% prevalence of RA, which is linked to the *1042-SE allele.[76]
This partly explains why a positive family history of RA increases the risk of developing RA.[77] The effects of these shared epitopes remain uncertain, but it is hypothesized that T-cells become activated when binding to the SE-MHC II receptor, co-initiating the inflammatory process.[78]

Not only CD4+ T-cells, but also other immune cells like macrophages invade the hypertrophic synovium, forming lymphoid aggregates and releasing proinflammatory cytokines. The majority of T-cells found in the synovium migrate from the bloodstream, rather than originating from local proliferation.

These findings are in line with findings that T cells found in the joint seem to have rather low proliferation rates.[79] The synovium in RA exhibits a high concentration of CD45+-T cells, reflecting a characteristic feature of T memory cells. It is assumed, that an antigen-dependent activation of T-cells promotes migration of immune cells into the synovium, and subsequent activation leads then to hyperproliferation and transformation of synovial fibroblasts.[76]
Taken together, RASF play a crucial role in RA pathogenesis,[80] and not only in direct response to an inflamed environment.

RASF are actively contributing to the perpetuation of RA:

1. RASF actively invade into adjacent structures like cartilage and also bone
2. RASF show uncontrolled proliferation and reduced apoptosis rates[66,81]
3. RASF maintain their aggressive behavior even in the absence of other immune cells and proinflammatory environment
4. RASF secrete immunomodulatory factors.

This further illustrates, why RASF are not only an important cell type for research of RA pathogenesis, but a promising therapeutic target for the complementary treatment of RA.

RA in the context of the endocrine and the nervous system

In recent years, there has been an increasing amount of research investigating the relationship between the immune, endocrine, and nervous systems.
Previously, these systems were considered to be separate entities in terms of anatomy, research focus, and function. However, recent and growing evidence suggests that they have a significant influence on each other.
For instance, neurotransmitters such as dopamine, once thought to be exclusive to the central nervous system (CNS), have been found on peripheral immune cells.[82] Additionally, it has been found that both male and female RA patients exhibit markedly reduced concentrations of androgens in their serum and synovial fluid, as compared to healthy control subjects.[83]

Several studies have investigated the role of the autonomic nervous system in rheumatoid arthritis (RA). They indicate a severe imbalance of the autonomic nervous system, both sympathetic and parasympathetic.
Patients with juvenile rheumatoid arthritis[84] (JIA) and rheumatoid arthritis (RA)[85] demonstrate elevated resting heart rates and increased urinary concentrations of metabolized catecholamines (3-hydroxy-4-phenoxyphenylglycol) compared to healthy controls, with no observed differences in blood pressure.

During the tilt up test (orthostatic stress test), it was observed that after 1 minute, cardiac output and diastolic blood pressure were lower than in healthy controls. Moreover, in patients with active JIA, norepinephrine levels did not increase to the same extent as in healthy controls after the test.[84] Studies have also demonstrated that individuals with RA exhibit decreased heart rate variability.[86] Additionally, the release of cAMP after β2 adrenergic activation on PBMC (peripheral blood mononuclear cells) was significantly lower in patients with active JIA than in healthy controls.[84]

Of note, the sympathetic nervous system appears to exert pro-inflammatory effects during the early stages of arthritis, but it seems to have anti-inflammatory effects during later or established stages.[87,88]
On the other hand, the parasympathetic nervous system has been shown to have anti-inflammatory effects, independent from the stage of arthritis.[87] In mice, for instance, activation of nicotinic acetylcholine receptors via vagus nerve stimulation results in significant amelioration of experimentally induced arthritis.[89,90]
Similar effects were observed in patients with RA. Vagus nerve stimulation via

an implanted electrical device resulted in a significant decrease in TNFα levels up to 84 days after stimulation. Additionally, the DAS28-CRP score showed significant improvement.[91] It is noteworthy that the anti-inflammatory effects of the parasympathetic nervous system are not limited to arthritis. Acetylcholine has been shown to inhibit the secretion of TNFα, IL1β, IL6, and IL8 in experimental models of endotoxemia,[5] bacterial peritonitis,[92] and acute hypovolemic hemorrhagic shock.[93,94] It was therefore called the cholinergic anti-inflammatory pathway.[95]
Further studies have shown that α7Ach-receptors on both immune cells and SF[96,97] play a crucial role in regulating anti-inflammatory effects.[5] In fact, in α7nAChR -/- mice, both the incidence and severity of arthritis were increased, as well as synovial inflammation and joint destruction aggravated.[98]

The sympathetic system serves as the counterpart to the parasympathetic nervous system. Research indicates that RA patients experience elevated sympathetic tone,[99] resulting in reduced heart rate variability, which may contribute to the higher incidence of cardiac diseases among those with RA compared to healthy control populations. There are numerous unknown parts about the role of the sympathetic nervous system in inflammatory processes, particularly in relation to RA. Recent research indicates that the sympathetic nervous system generally induces a shift in the immune response from Th1-dominant to Th2-dominant. For instance, research demonstrates that norepinephrine and epinephrine have the ability to significantly reduce the production of proinflammatory cytokines such as IL12, TNFα, IFγ and, in turn, promote the production of anti-inflammatory cytokines such as IL10 and TGFβ.[100]

Moreover, the sympathetic nervous system can regulate leukocyte migration towards wound sites after surgery. This ability can be increased up to 300% when mice are stressed prior to surgery.[101]
Although the sympathetic nervous system is associated with ambiguous effects on inflammatory processes, sympathetic nerve fibers seem to disappear from the synovium in RA patients but not in OA patients during the course of the disease.[102] Synovial inflammation and the loss of sympathetic nerve fibers are negatively correlated.[102]
This observation can also be seen in Crohn's disease, another autoinflammatory disease.[103] Recent studies indicate that the reduction of sympathetic nerve fibers is caused by semaphorin 3C, which is highly expressed in patients with RA, but not OA, and is known to be a sympathetic nerve repellent.[104]
As the sympathetic nerve fibers decrease, the amount of Substance P posi-

tive nerve fibers increases.[102] Among others, one main effect of Substance P is the transmission of pain and increase of TNFα[105] and IL1[106] levels. Inhibition of Substance P results in substantial reduction of inflammation and an has chemo-attractive effects on monocytes.[107]

But even when sympathetic nerve fibers decrease, tyrosine hydroxylase-positive cells (TH+ cells) like macrophages (CD163+), lymphocytes[108,109] and SF (prolyl-4-hydroxylase+) are still present in the synovium and contribute significantly to the local catecholamine production[110] of epinephrine, norepinephrine and dopamine.[108,111] This explains, why released norepinephrine levels from RA and OA synovial cells do not significantly differ.[111] The amount of TH+ synovial cells and also VMAT2-positive cells are significantly higher in RA than in OA.[111,112] VMAT2 (vesicular monoamine transporter) is a transporter protein located in the membrane of intracellular dopamine-storing vesicles.

Furthermore, blocking VMAT2 by using reserpine leads to an extracellular increase of catecholamines by leading to exocytosis of all catecholamine-storing vesicles.[113] By treating synovial cells from RA and OA patients with reserpine, a significant dose-dependent decrease of TNFα levels could be observed.[111] Additionally, significant amelioration of paw inflammation in mice with collagen type-II arthritis was achieved by local application of reserpine.[111]

Taken together, the parasympathetic and sympathetic nervous system seem to play a pivotal role in RA pathogenesis.

1.1.3 Therapy of RA

Up to the present day, there is no cure for RA. Looking back less than a hundred years ago, intravenous gold injections were a common therapy for RA. Gold was frequently used in the 1950s and 1960s, although it has little beneficial effect on patients. In some countries, it is still used today. Fortunately, therapeutic options have significantly improved today. In terms of medication, several types of drugs have been demonstrated to improve patients' quality of life and slow down the progression of joint destruction. These substances are termed as "disease-modifying antirheumatic drugs" (DMARDs).

Generally, DMARDs can be categorized into three groups: conventional synthetic DMARDs (csDMARDs), biological DMARDs (bDMARDs) and targeted

synthetic DMARDs (tsDMARDs).[114]
The primary agents of conventional synthetic DMARDs are methotrexate (MTX), leflunomide, sulfasalazine, azathioprine, and hydroxychloroquine.
Among these substances, methotrexate (MTX) is the first-line csDMARD,[115] as it has been shown to efficiently slow down joint damage as well as improve quality of life[54,116] along with good and fast response rates. Accordingly, MTX treatment ought to be initiated immediately after diagnosis of RA has been established.
Originally, methotrexate was utilized as a chemotherapy drug in cancer treatment, functioning as an antagonist to folic acid. In the treatment of rheumatoid arthritis, however, it is administered in far lower doses than in the treatment of cancer to reduce only the activity of immune, proinflammatory cells and fibroblasts. When combined with glucocorticoids, methotrexate leads to rapid symptomatic relief, but side effects can occur with high-dose use.
Alternatively, Leflunomide and Sulfasalazine can be used. Sulfasalazine, for example, is often used during pregnancy. Taken together, the csDMARDs are a group of drugs that have different and multiple modes of action. The beneficial effects on disease activity and the precise biochemical effects of csDMARDs have been investigated over time, after they were established and used widely in RA therapy. This is one significant point that distinguishes csDMARDs from biological DMARDs. Biological DMARDS operate via specific, defined molecules in activated inflammatory pathways. Infliximab and Etanercept were the first bDMARDs to be approved for the treatment of RA.

Infliximab, a chimeric human/mouse monoclonal antibody, binds to TNFα and neutralises it, thereby preventing inflammation from continuing and exacerbating. In contrast to csDMARDs, Infliximab is a protein-based drug, as it is an artificially produced antibody. But not all bDMARDs are antibodies: Etanercept is a specific receptor for TNFα, and hereby also eliminates circulating TNFα. Of note, studies have demonstrated that when first-line MTX therapy fails, step-up therapy with bDMARDs is more effective than a second csDMARD therapy.[117]

Biological DMARDs have been used in RA therapy for about 20 years, and similar substances to the "original" biological DMARDs have been developed. These new molecules are structurally similar, but not completely identical, to the original biologic DMARD. Nevertheless, the pharmacological target remains the same. They are subsumed as biosimilar DMARDs (bsDMARDs). In 2013, the first bsDMARD, an infliximab biosimilar, received approval. Inflectra® is also based on proteins, like the original biological DMARDs.

Biological DMARDs were the first drugs in RA therapy with a specific target. Central inflammatory pathways have been identified and then used to identify respective therapeutic targets. A comparable approach was used in the development of targeted synthetic DMARDs (tsDMARDs), also known as "small molecule drugs". These drugs also act through crucial inflammatory pathways. However, similar to csDMARDs, they have a molecular structure that is synthetic and not protein-based. This has a notable advantage in clinical practice as these drugs, like most csDMARDs, can be taken orally. In contrast, biological DMARDs must be administered intravenously or subcutaneously because the protein structure needs to be preserved. In 2017, baricitinib (a tyrosine kinase inhibitor) as the first tsDMARD was approved for RA therapy in Europe. Shortly after, another tyrosine kinase inhibitor, tofacitinib, was also approved. It targets janus kinase 1 and 3, which are key checkpoints in inflammatory pathways, resulting in a significant decrease in proinflammatory cytokines such as IL6.[118]

Tofacitinib was found to be superior to MTX monotherapy in clinical symptoms and radiographic progression of joint destruction. In addition to DMARD therapy, corticostroids are also a key players in the treatment of RA, particularly during acute phases. They are frequently prescribed in lower doses alongside established DMARD therapy during periods of reduced disease activity. Given as a inflammatory compound, it not only reduces inflammation but also significantly improves morning joint stiffness, thereby contributing to patients' well-being. Whereas acute phases of disease require higher doses of cortisone, low-dose therapy or even stop of cortisone therapy are the long-term goal in phases of remission. Of note, cortisone therapy has only a limited effect on RA-caused joint destruction. The three types of DMARDs used for RA therapy differ and have evolved over time, but all drugs are used to achieve the same goal: disease remission. The timing of treatment initiation is crucial, as early intervention is essential, in addition to the type of medication. Additionally, aside from drug therapy, manual therapy and lifestyle changes such as a healthy diet and stop smoking have a positive and significant effect on the course of the disease.
Physiotherapy and also psychological support are also essential components for improved life quality. In order to maintain remission and adjust treatment, monitoring scores like the Disease Activity Score (DAS-28) or Clinical Disease Assessment Index (CDAI) and Simplified Disease Assessment Index (SDAI) should be repeatedly performed.
This ensures that treatment goals are met and enables early intervention when

symptoms worsen. Inadequate responses on drugs should be assessed and as a consequence, treatment has to be adjusted.

1.2 Osteoarthritis

In contrast to RA, OA is not an autoimmune disease, but a disease that is caused by increased wear of the joints over the years.
This is why OA primarily affects weight-bearing joints such as the hips and knees, but also the thumbs and shoulders. There are two types of OA: primary osteoarthritis, which occurs without any apparent external influence due to advanced age, and secondary osteoarthritis, which is a long-term consequence of other conditions such as obesity, previous trauma, repetitive inflammation or inherent or acquired joint malposition.[119]

As malpositions such as genu valgum and varum lead to uneven weight bearing and subsequent overloading of certain areas of the articular surface, OA can sometimes be observed only in certain parts of the joint. During the initial stages of OA, patients often report "warm-up pain" that diminishes during the day, and pain that aggravates with activity (e.g., running).
As OA progresses, pain often persists throughout the day, and joint mobility decreases over time, causing physical limitations. As cartilage and, in advanced stages, also bone gets lost, leg length differences can occur. This results in increased weight-bearing on the opposite leg and may cause back pain due to the spinal column's misalignment.
This is why already early stages of OA should be under frequent reevaluation and, if necessary, be treated for example with orthopedic insoles.

X-rays reveal typical signs of OA, including joint space narrowing due to cartilage loss, subchondral sclerosis, osteolysis, and osteophytes. Kellgren and Lawrence[120] established an internationally accepted scoring system for OA, which can be applied in a standard x-ray. The presence of osteophytes, joint space narrowing, subchondral sclerosis with pseudocystic areas and sclerotic walls, and joint or bone deformity are the criteria used to identify the stages of osteoarthritis.
By these criteria, stages 0 to 4 can be distinguished (Table 1.2). Osteoarthritis is common, and although it is not possible to regenerate lost cartilage, there are several surgical and non-surgical treatment options. The goal is to minimize pain and loss of function of the joint and to prolong arthroplastic surgery.

Although arthroplasties have made significant progress in recent decades, it is still ultima ratio in OA treatment. Proper pain management, physical therapy, and personalized orthotic devices are fundamental techniques for preventing joint arthroplasty or delaying its implementation.
Pain and inability to handle daily life often require strong pain medication and finally endoprosthetic replacement of the joint. Although OA is not considered a typical inflammatory disease, inflammation in the affected joints is common.[121] However, this inflammation is a response to overuse and the joint's inability to cope with the demands caused by ongoing deterioration over time, in contrast to rheumatoid arthritis.

Table 1.2
Kellgren and Lawrence Score[120,122]

Stage	Radiographic findings
0	No radiographic alterations of osteoarthritis
1	Doubtful narrowing of joint space Possible osteophytes
2	Osteophytes Possible narrowing of joint space
3	Moderate presence of osteophytes Definite narrowing of joint space Small pseudocysts with sclerotic walls Possible deformity of bone contour
4	Large osteophytes Vast narrowing of joint space Severe sclerosis Definite deformity of bone contour

Kellgren and Lawrence established a scoring system for osteoarthritis by radiographic criteria like joint space narrowing, presence of osteophytes, pseudocysts and sclerotic walls and bone deformity are evaluated. This classification is limited to radiographic findings, clinical symptoms do not count.

1.3 Dopamine

Belonging to the family of catecholamines, dopamine plays a substantial role both inside and outside the central nervous system (CNS). Within the CNS, dopamine is involved in motor control, the brain reward system, and cognition. Beyond the CNS, dopamine plays a key role in blood pressure regulation, gastrointestinal motility, and immune function. Dopamine (DA) receptors are present in nearly every organ,[123] highlighting their crucial involvement in various pathways and tissues throughout the body.

1.3.1 Structure and synthesis of dopamine

The non-essential amino acid tyrosine serves as the initial substance for catecholamine synthesis.
In the first step of dopamine production, which is rate-limiting, tyrosine hydroxylase oxidizes L-tyrosine to L-dihydroxyphenylalanine (DOPA, Fig. 1.7).[124] This process requires tetrahydrobiopterin (BH4) as a cofactor. As both catecholamines and BH4 compete for iron ions, further catecholamine production is self-limiting (negative feedback loop).[125] Tyrosine hydroxylase activity and thus

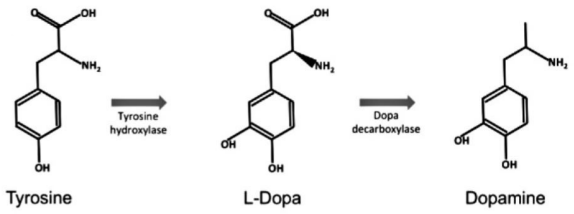


Figure 1.7
Synthesis of dopamine[126] from tyrosine, a non-essential amino acid. In the first, rate-limiting step of dopamine production, L-tyrosine is oxidated by the pacemaker enzyme tyrosine hydroxylase to L-dihydroxyphenylalanine (DOPA).[124] Therefore, tetrahydrobiopterin (BH4) is needed as a cofactor. In the final step of dopamine synthesis, DOPA is decarboxylated to dopamine.[124]

dopamine production can increase by elevated intracellular cAMP levels,[127] but also by phosphorylation of tyrosine hydroxylase.
This usually results in an enhanced enzyme activity.[128] Furthermore, hypoxia is often present in inflamed tissue and has been shown to increase TH activity. Dopamine levels have been found to be higher in cells treated under hypoxia when compared to normoxia treated cells.[129,130] Finally, DOPA is decarboxylated to dopamine in the last step of dopamine synthesis.[124]

Once dopamine synthesis is complete, the neurotransmitter is either stored in vesicles for later use or further processed into norepinephrine by dopamine-β-hydroxylase. Storing dopamine in intracellular vesicles protects the cell from oxidative stress, as increased dopamine degradation leads to increased levels of reactive oxygen species (ROS). Using this pathway, the cell protects itself from the toxic ROS effects by separating dopamine.[131] Vergo et al. demonstrated that transfection of VMAT2 in dopaminergic cell lines (PC12; these cells are phaeochromocytoma cells of rat adrenal medulla) reduces cell death significantly.[131]
After the release of dopamine, surplus extracellular dopamine can be taken up by dopamine transporters (DAT) in symport with sodium.[132] Dopamine is then restored to vesicles until its next use. As a result, dopamine transporters play a significant role in dopaminergic effects.[133]
Dopamine transporter activity can be influenced by different pathways: for example, protein kinase C (PKC) activation leads to lower DA transporter activity[134,135] via phosphorylation,[136] which also increases internalization of DAT.[137] Subsequently, this leads to higher extracellular dopamine concentrations. Additionally, inhibition of the MAPK pathways in HEK293 cells considerably reduces DA uptake.[138]
Activation of D2DR results in an increase in dopamine reuptake, while inhibiting D2DR has the opposite effect.[139,140] The proinflammatory arachidonic acid was shown to inhibit DA uptake and led to increased dopamine release.[141] Modifying DAT function and also its expression on the cell surface is therefore an elegant way to modulate DA effects.

Dopamine molecules that are not stored in vesicles following synthesis or reuptake undergo an enzyme cascade leading to their degradation to homovanillic acid. The most frequent way in which dopamine is metabolized is by the enzyme monoamine oxidase (MAO).
Even though most research focuses on dopamine production and effects, elimination pathways are also interesting. As mentioned before, high concentrations of dopamine and subsequent degradation lead to increased levels of hydrogen peroxide and dihydroxyphenylacetic acid.[142] These metabolites in turn lead to severe oxidative stress in the cell. Further degradation happens then via alcohol and aldehyde dehydrogenases to homovanillic acid.

Another possibility to deactivate dopamine is via catechol-O-methyltransferase (COMT). The COMT pathway is not specific for dopamine degradation, but a rather common pathway for catecholamine deactivation especially outside the

CNS.
Taken together, dopaminergic effects are not only dependent of DA production. In fact, DA synthesis is only one of many components in order to achieve proper dopaminergic effects.

1.3.2 Dopamine receptors

In principle, dopamine receptors can be divided into five receptor subtypes belonging to the family of G-protein coupled receptors (GCPR).[143] Because of their typical 7-fold transmembrane structure, they are also called heptahelical receptors.[144]
As the name suggests, extracellular binding of dopamine to a respective dopamine receptor leads to intracellular activation of a coupled G-protein. This is a heterotrimeric protein composing of an alpha (α), beta (β) and gamma (γ) subunit.
After the activation of the G-protein using GTP, it dissociates into the α subunit as one part and the β and γ subunit as another part. Both the α and β/γ subunits can now activate or inhibit other proteins and pathways. The five subtypes of dopamine receptors can be categorized into two receptor subgroups corresponding to their respective intracellular effects (Fig. 1.8). The D1-like receptor subgroup comprises dopamine receptor subtypes 1 and 5, known as D1DR and D5DR. These receptors operate intracellularly through G$\alpha_{s/olf}$ signaling(Fig. 1.8). The extracellular activation of D1-like receptors leads, as described above, to a dissolvement of the activated G-Protein and subsequent activation of adenylatcyclase by the α subunit. This enzyme now produces cAMP, which serves as the second messenger of G$\alpha_{s/olf}$.

By contrast, binding of dopamine to a D2-like receptor, including dopamine receptor subtype 2 (D2DR), subtype 3 (D3DR) and subtype 4 (D4DR), leads to an activation of G$\alpha_{i/o}$ proteins.[145,146] When the heterotrimeric G-protein is activated and dissolved, it inhibits adenylate cyclase, resulting in a decrease of intracellular cAMP concentration. In summary, activation of dopamine receptors can either increase or decrease intracellular cAMP, depending on the respective GPCR subtype activation.
Elevated cAMP levels impact various pathways, such as the ionic glutamate receptor (NMA and AMPA) (Greengard, 2001), and further activate protein

kinase A, which in turn has a number of effects, such as altering CREB-dependent gene expression.[147] However, further investigation has shown that dopamine receptor effects are quite complex and go well beyond G$\alpha_{s/olf}$ and G$\alpha_{i/o}$ signaling.

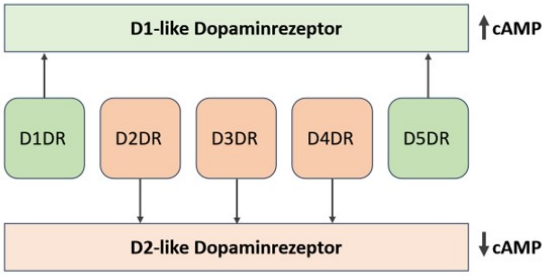


Figure 1.8
There are five different subtypes of dopamine receptors. All of these subtypes of dopamine receptors act through G protein-coupled receptors. D1DR and D5DR comprise the D1-like receptor subtype, acting through G$\alpha_{s/olf}$ pathways within the cell, which leads to an increase in intracellular cAMP concentration. D2DR, D3DR, and D4DR form the D2-like receptor subtype. They act through the G$\alpha_{i/o}$ pathway and reduce the intracellular cAMP concentration after activation.
(Source: Own illustration)

Recent research showed, that beside the well-known above-mentioned pathways, dopamine also operates via Gq-protein coupled receptors and subsequent activation of phospholipase C, which in turn induces calcium release via IP3 receptor activation.[148,149]
Additionally, dopamine seems to have multiple GPCR-independent effects:[150,151]
Dopamine was demonstrated to directly regulate calcium channels, for example via D2DR,[152] and also inward directed potassium channels.[153]
Both D1DR and D2DR subtypes have been shown to interact with the Na^+K^+-ATPase.[154,155] Interestingly, function of D1DR and D2DR is also dependent on the expression of the Natrium-Kalium-ATPase in brain cells. As Hazelwood et al. could show, overexpression of Natrium-Kalium-ATPase leads to lower density of D1DR and D2DR, and inhibition of Natrium-Kalium-ATPase increased D1DR and D2DR function in both brain and HEK293T cells.[154]

Dopamine receptors are also capable of forming heterodimers with other DRs, such as D2-D4 heterodimers, which act intracellularly via MAP kinase pathways,[156] or D1DR-D2DR heterodimers in striatal cells,[157] which act via a $G\alpha_{q/11}$-coupled pathway.[158] Similar signaling cascades have been shown to follow on coactivation of D5DR-D2DR receptor hetero-oligomers.[159]

Protein kinase C was found to cause phosphorylation, internalization, and desensitization of D2DRs[160] and D3DRs.[161] Further, the phosphorylation[136] caused by protein kinase C activation is linked to decreased activity of the DA transporter[134,135] as well as increased internalization of dopamine transporters.[137]

Adenosine receptors also frequently partner with dopamine to form heterodimers. Among these, the adenosine receptor subtype A2A is one of the most extensively studied.[162–166] These heterodimers seem to be dynamic. As Gines et al showed, the heterodimerization between Adenosine A_1 receptors and D1DR disappeared after pretreatment with the D1DR agonist in mouse fibroblast Ltk^- cells[167] and on the other hand treatment with Adenosine A_1 receptor agonists led to a coaggregation of Adenosine A_1 receptors and D1DR.[167]

In summary, dopamine receptor signaling remains a highly complex issue that has yet to be fully comprehended and explored. Additionally, it remains uncertain whether the effects of dopamine receptor activation and heterodimers are identical in the periphery as in the central nervous system.

1.3.3 Role of dopamine

For a long time, dopamine was thought to play its main role in the central nervous system. It is responsible for essential cognitive functions like movement and emotion regulation, as well as the regulation of other hormones like prolactin.
Additionally, dopamine plays a crucial part in the brain's reward system. An impaired dopamine metabolism is known to be involved in several neurological diseases such as Parkinson's disease, schizophrenia, depression, drug addiction or attention deficit hyperactivity disorder. The symptoms observed in these diseases illustrate the importance of a well-running dopaminergic system.

Of interest, these disorders are often accompanied by changes in immune cell counts and function.[168] In patients with Alzheimer's disease, expression of D2DR on peripheral blood lymphocytes is significantly lower compared to healthy controls.[169] Similar results have been observed from patients with Parkinson's disease: here D3DR density was significantly lower compared to control group.[170] By contrast, untreated patients with schizophrenia were shown to have significantly higher expression of D3DR mRNA in T-cells.[171] Dopamine receptors have also been identified on most immune cells such as B cells.[172]
Moreover, research indicates a negative correlation between Dopamine D2-receptor levels in the brain and body mass index (BMI) in obese patients.[173]

Over the past few years, it has become evident that dopamine plays a significant role also beyond the blood-brain barrier. As dopamine cannot cross the blood-brain barrier itself, dopamine metabolism is often divided into "central" and "peripheral" dopamine metabolism. Dopamine is typically classified as a neurotransmitter; however, there are dopamine receptors in almost every organ in the human body. In intensive medical care, for example, dopamine has been used for a long time in order to improve heart function and regulate blood pressure. Dopamine is also thought to be involved in development of hypertension by influencing renal blood flow and natrium homeostasis.[174]
Further effects of dopamine contain regulation of endocrine pancreatic hormones. Increased levels of dopamine significantly reduce insulin release.[175] This effect could also be observed in patients with Parkinson's Disease: At the beginning of L-DOPA treatment, insulin levels in patients' blood serum are significantly decreased.[176]
Moreover, it regulates homeostasis via alternation of von Willebrand factor

release, circadian rhythm via dopaminergic signaling in the retina[177] and gut motility.[178] Even in cancer cells, dopamine has notable effects by stimulating cell proliferation in non-cancerous cells while impeding cell growth and proliferation in cancerous cells.
In mouse models of colon cancer, the efficacy of anticancer drugs administered is enhanced when dopamine is added.[179] This might be due to dopamine's inhibitory effects on neoangiogenesis.[180] Besides the well-known effects of dopamine, it is also thought to play a significant role in the development of cancer: in patients with schizophrenia there is a significantly lower incidence of colon and lung cancer.[181]

Even stem cells are impacted by dopamine. Specifically, in human CD34+ cord blood cells that were pretreated with G-CSF, dopamine significantly increased migration during a transwell assay.[182] Taken together, dopamine is involved in several physiological pathways and diseases like cardiovascular events, obesity, cancer and inflammation.
The impact on inflammation by dopamine receptor activation has been further investigated during the last few years with interesting results.

Dopamine and the immune system

Over the past few years, there has been increasing evidence of the impact of dopamine on the immune system. However, as more research is conducted in this field, the effects of dopamine appear increasingly complex and far from obvious. This is also true for dopamine receptor expression on immune cells, which is highly dependent on cell differentiation subset and activation state.[148] Dopamine receptors have been documented to be expressed on most immune cells.[183]

At first, dopamine receptors have been described on T-cells in 1980.[184] Depending on the cell type or even differentiation subset, dopamine receptor expression is different and not all receptor subtypes can be found on immune cells. For example, neutrophils do not express D1DR, but mainly D3DR and D5DR, and also D2DR and D4DR in lower concentrations.[172]
Also, the expression of some dopamine receptor subtypes sometimes changes after cell activation: For instance, D3DR is the predominant dopamine receptor subtype found on naive CD8+ T cells.[185] As demonstrated by Watanabe et al., the expression of D3DR was markedly reduced following T-cell activation.[185]
At the same time, most immune cells are positive for tyrosine hydroxylase and

therefore produce dopamine by themselves.[186–189]

Regarding whether dopamine effects are pro- or anti-inflammatory, it appears that dopaminergic effects are highly dependent on the dosage. Low concentrations are believed to be immunoactivating, while high local concentrations of dopamine seem to have immunosuppressive effects.[190]
The findings of Cosentino's group confirmed that low doses of dopamine (100-500 µM) decreased intracellular ROS levels and inhibited apoptosis via D1-like receptors in lymphocytes. Conversely, high doses of dopamine significantly increased intracellular ROS concentrations and apoptosis rates. These proapoptotic effects of dopamine on lymphocytes in high concentrations are consistent with findings from other groups.[191–193]

As local dopamine concentrations highly differ between different organs and are also altered in different states of disease, it is very difficult to conduct experiments with dopamine in physiological concentrations.[148,190]
Results in favor of an immunosuppressive effect of dopamine show that for example phagocytic capabilities of neutrophils are reduced in a dose-dependent manner under incubation with dopamine. Additionally, expression of adhesion molecules like CD11b and CD18 and adhesion itself was shown to be reduced under dopamine treatment.[194] Sookhai et al showed concordant results with significantly reduced migration of neutrophils in a transwell assay under stimulation with dopamine.[195]
Regarding T cells, migration and adhesion of T regulatory cells were shown to be significantly reduced after D1DR activation compared to untreated cells by decreased CTLA4 expression and ERK phosphorylation.[196,197] Suppressive effects on both activated and non-activated T-cells by bromocriptine, a D2DR-agonist, were also observed.[198,199] Besides, already activated T-cells show a reduced differentiation and proliferation after treatment with dopamine.[200,201]

On the other hand, there are results that support a rather proinflammatory role of dopamine. For instance, the work-group of Levite observed an activation of resting T-cells via a specific D2DR and D3DR activation and also a significant increase of T-cell migration and extravasation.[202]
Similar results were obtained by Watanabe in 2006 with promoted migration of human and mouse CD8+-T-cells after specific D3DR activation.[185] Regarding cytokine release, results are also ambiguous. Whereas the induction of proinflammatory cytokine release like TNFα by dopamine was reported by some authors,[203–206] a significant decrease of IL 6 and IL 8 release from

RASF was shown by others.[197,207] Reversely, proinflammatory cytokines were shown to decrease extracellular dopamine levels by increasing the expression of dopamine transporters on the cell surface.[138]

Concerning cell proliferation, dopamine seems to have rather suppressive effects,[190,208,209] which could also be shown by using L-DOPA, the precursor molecule of dopamine.[210]
Apart from that, dopamine has immunoregulatory effects by shifting the immune response towards a Th2- and Th17-response in CD4+ naive T-cells after activation.[124] Supplementing these results, Ria et al showed, that dopamine successfully suppressed the release of IL12 p70 from neutrophils. IL12 p70 is an important cytokine for Th1-induction in dendritic cells.[211]

Taken together, effects of dopamine on the immune system are manifold. A variety of acute, chronic and autoimmune diseases are currently being studied in order to elucidate dopaminergic effects on the immune response. In particular, the role of DA in the pathogenesis of autoimmune diseases remains to be determined, but promising results have been achieved in the field of rheumatoid arthritis in recent years.

Dopamine in RA - what do we know?

As dopamine began to come into focus as a mediator of inflammatory processes, some attention fell on autoimmune diseases. In the context of rheumatoid arthritis, there are some links to other diseases that suggest an important role for dopamine.
For example, it was shown multiple times, that patients suffering from schizophrenia are less likely to develop RA than others.[212–214]
The pathogenesis of schizophrenia is thought to be the result of a dysfunction in dopaminergic metabolism.[213] Similar is also suspected for causing Restless-Legs-Syndrome (RLS). Here, a strong association between RA and RLS has been observed.[10] Whereas schizophrenia is thought to be caused by a pathological increased dopamine metabolism, RLS is thought to be caused by dopaminergic hypofunction.[215]
Therefore, it is commonly treated with dopamine agonists. Also, patients with RA have higher dopamine levels in their blood serum than healthy controls.[216]
This is also true for DA levels in their synovial fluid: DA levels are significantly higher compared to patients with for example OA. Other catecholamine serum levels like adrenaline and noradrenalin were not altered, though.[217] These find-

ings put dopamine into focus as a potential crucial player in RA.
When investigating B-cells from RA patients, expression of D2DR is lower compared to healthy donors. More, D2DR expression is negatively correlated with CRP-levels in blood serum and clinical disease activity indices like DAS28 and SDAI. Additionally, the expression of D2DR, D3DR and D5DR are also negatively correlated with RF levels in serum.[218]
In animal experiments, D2DR-activation with quinpirole significantly improved arthritis in mice with collagen induce arthritis (CIA).[219] On the other hand, CIA-D2DR-knockout (CIA-D2DR-/-) mice suffered from significantly more severe symptoms and course of arthritis.[219,220] In turn, administering D1DR-agonists and carbidopa to CIA-mice significantly ameliorated arthritis symptoms.[221,222]

Interesting results were also obtained by Nakano et al. They transferred RA synovial tissue and cartilage into human RA/SCID chimera mice. The mice then received either a specific D1-like (SCH23390) or D2-like (haloperidol) receptor antagonists or vehicle (as a control group). After 30 days, the group treated with D1-like receptor antagonists showed a significant reduction of before hyperproliferated synovium, whereas mice treated with the D2-like antagonist showed growing synovial tissue combined with increased vascularization. Additionally, whereas the SCH23390 (D1-like receptor antagonist) group had only slight cartilage destruction, this was increased under treatment with haloperidol,[217] an D2-like dopamine receptor inhibitor. In rats with experimentally induced arthritis, haloperidol was able to significantly reduce serum levels of rheumatoid factors as well as MMP3 and CRP levels. Regarding the joint cartilage surface, these rats showed an even cartilage surface without erosions, in contrast to the untreated rats under haloperidol treatment.[223] Dopamine is also able to increase IL1 production in human naive CD4+T-cells.[217]
Besides leukocytes, synovial fibroblasts were also shown to be able to produce (by expressing tyrosine hydroxylase), store and release dopamine and are also reactive to extracellular dopamine by expressing dopamine receptors and transporters.[111,207] Interestingly, this could not be observed in other fibroblasts like skin fibroblasts.[224]

When examining the synovium of RA and OA patients, it is remarkable, that cells positive for tyrosine hydroxylase and also L-DOPA are significantly more frequent than in OA patients.[111] Also, all DR subtypes were found on SF from both RA and OA patients.[207] DR-density on the cell surface and mRNA levels were higher in RA patients than in OA patients.[207] In RASF and OASF cell cultures, dopamine was shown to be able to reduce IL6 and IL8 levels also in

both RA and OA patients, although effects were stronger in RA.[207] The inhibition of dopamine storage by blocking VMAT2 with reserpine, led to significant amelioration of arthritis in CIA mice together with a remarkable reduction of TNFα release in a dose-dependent manner.[111]

Although experimental outcomes are very promising, attempts of treating RA with DR agonists led to unsatisfying and sometimes contradictory results.[225,226] As a lot of questions are still left open, further investigation towards specific receptor subtypes and their respective effects on disease pathogenesis is needed to find a specific therapeutic target.

1.4 Aging in the context of RA

Aging is a natural process in life. However, its effects are manifold. When observing older people, "symptoms" such as presbycusis, osteoarthritis, muscular atrophy and dementia are the most common.
Those "symptoms" are oftentimes summarized as "frailty". Therefore, so-called frailty-scores are commonly used in geriatric assessments.
Taking a closer look at the cellular level of the natural aging process, it becomes apparent that it encompasses more than "just" hearing loss or forgetfulness. Especially the immune system is affected from this process.

With increasing age, immune responses get more and more dysregulated.[227] Elderly individuals were found to have increased baseline pro-inflammatory activity[228] and an impaired response of the adaptive immune system.[227] Sensitivity of T-cell receptors and therefore the ability of T cells to react properly to pathogens is significantly reduced after the age of 70.[229]
Similar things have been shown for the sensitivity of adrenergic receptors, which is significantly reduced with increasing age.[230] The migration of neutrophils to sites of inflammation is a critical feature of neutrophil function. However, with increasing age, this migration undergoes significant impairment. In particular, neutrophils from aged mice were not shown to migrate slower, but they respond significantly less to a migration-inducing stimulus like IL8.[231–233] Besides, neutrophil chemotaxis was also decreased.[231]

Regarding skeletal muscle cells from aged mice, they migrate significantly slower than from younger mice.[234] These findings (among others) established the term "inflamm-aging", pointing out the significant changes that the immune system is undergoing during aging.[235]

As previously stated, the cause of RA is still not fully elucidated. According to one of several current theories, RA develops because of premature aging of the immune system.[236,237] T-cells from patients with rheumatoid arthritis exhibit a significant reduction in diversity when compared to those from healthy controls.
Specifically, a 50-year-old RA patient displays a T-cell signature resembling that of a healthy individual of 70 to 80 years old.[238] Telomer length is an important and well-established parameter for cell aging.[239] Interestingly, when measuring the telomer length in RA patients, they are significantly shorter than those from healthy controls.[240,241]
The group of Cornelia Weyand investigated this phenomenon with interesting results. The HLA-DR4 allele is also strongly associated with the development of RA. They found out, that in cells obtained from cord blood of HLA-DR4+ and HAL-DR4- babies, telomere lengths were not altered. However, when analyzing telomer length at age of 20, telomers of healthy HLA-DR4+ individuals were significantly shorter than from healthy HLA-DR4- ones.[241,242]
However, in HLA-DR4+ patients with RA, shortening of telomers in T-cells was not associated with disease progression. The authors assumed then, that this rather affects the predisposition for RA than influencing the course of disease.[241] Increased age represents the most significant risk factor for developing RA.[243] Additionally, the older the patient is at the onset of RA, the longer RF can be found in serum before disease onset.[244]

Synovial fibroblasts were also shown to be prematurely aged in RA.[245] The senescent marker p16 could be found in SF obtained from RA, OA and healthy controls. But the overall density of p16 positive cells was higher in RA and OA patients than in healthy controls.
In younger RA patients (< 40 years), the p16 expression was significantly higher compared to OA patients and healthy controls. Yet, in older RA patients, levels of p16 expression were similar to control groups.[245] Montero-Melendez et al. showed, that senescence in RASF can be promoted via GPCR activation.[246] Selective melanocortin-1 receptor activation (which is known to be a GPCR) led to significant increase of SF senescence via ERK1/2 pathway.[246]
Regarding dopamine receptors, a significant loss of all DR subtypes in the

brain[247–249] has been described with increasing age.
In line with this, dopamine transporter density and dopamine synthesis also decrease with age in both the central nervous system and the periphery.[250–254] Although several studies have demonstrated this phenomenon, the underlying mechanisms driving the downregulation of the dopaminergic system remain unclear.
This is also true for the phenomenon of "immuno-aging", even though this phenomenon is known for several years now, and more and more changes in cytokine profiles, effects and changes in immune responses are elucidated.

Chapter 2

Aims

Dopamine plays a critical role in inflammation and appears to be involved in the pathogenesis of rheumatoid arthritis. As synovial fibroblasts have an intrinsic dopaminergic system, this study aimed to explore the role and influence of dopamine in the pathogenesis of the disease, ficusing on three queastions:

1. How are different DR-subtypes distributed within the synovium?

   As the presence of dopamine receptors on RASF and OASF has already been described, the distribution of the respective DR-subtypes within the synovium was of special interest in this study.

2. Does DR-activation influence the aggressiveness of RASF and OASF as a control group?

   Invasion of RASF into the adjacent cartilage is a crucial process in the course of RA leading to joint destruction over time. As dopamine was shown to have promoting effects in T-cell migration, we now wanted to further investigate, whether this is also true for RASF. This is of special interest, as the inhibition of RASF migration into the adjacent cartilage and bone is an important feature for an effective cartilage-protective treatment.

3. Is cytokine release from RASF and OASF influenced by DR-activation?

   As it is still unclear whether dopaminergic effects are either pro- or anti-inflammatory in RA, the goal was to investigate whether specific D1-like or D2-like receptor activation leads to significant changes in IL6 or IL8 release. Further, we wanted to know, whether dopamine receptor activation has any effects on matrix degradation by increasing the release of matrix-degrading enzymes.

In the past, different and also opposite dose-dependent effects of dopamine have been described. This is why different concentrations of specific D1-like and D2-like receptor agonists were used.

Chapter 3

Materials and Methods

3.1 Patients

For the experiments, synovial fibroblasts from rheumatoid arthritis and osteoarthritis patients (OASF and RASF) were obtained during knee arthroplasty at the Markus Hospital in Frankfurt am Main, Germany. All patients gave written consent. This research project was ethically approved by the ethics committee at Justus Liebig Universität Gießen (file number 66/08).

Synovial fibroblasts from 31 patients with RA and 28 with OA were included in the experiments (Tbl. 3.1). The numbers of tissues and cells used in each experiment are indicated in the results section. In both groups, women were more frequent than men. Mean age was 63.3 years in RA with a range of 27.4 to 87.8 years and mean age of 74.4 years, and a range of 58.8 to 88.5 years in OA patients. For 19 of 31 RA patients, information about treatment with glucocorticoids and 11 with MTX was available. Two patients with OA were treated with systemic glucocorticoids for other reasons. Inhalative glucocorticoids were not included.

Table 3.1
Table of patients included into all experiments.

Characteristics	**RA** (n=31)	**OA** (n=28)
Age, mean (range) in years	63.3 (27.4-87.8)	74.4 (58.8-88.5)
Men/ women	3/28	9/19
Disease duration, mean (range) in years	16.4 (2-46)*	no data available
Glucocorticoid therapy	19*	2*
Methotrexate	11*	–
TNFα inhibitors	9*	–
Rituximab	2*	–
Leflunomide	2*	–

No data was available about disease duration in OA.
Information about medication was not available for all patients included into the study.

3.2 Materials

Table 3.2
Materials used for experiments

Materials	Company
12- and 24 well-plates	Greiner, Frickenhausen, Germany
Boyden Chamber	Neuroprobe, Gaithersburg, USA
Cell culture flasks (75 cm^2)	Corning, Wiesbaden, Germany
Cryotubes (2ml)	Greiner, Frickenhausen, Germany
Falcon tubes (15ml/50ml)	BD Biosciences, Heidelberg, Germany
Polycarbonate PVPF-membrane, 8 µm pore size	GE Osmonics, Minnetonka, USA
SuperFrost Plus slides	Menzel Gläser, Braunschweig, Germany

Table 3.3
Chemicals used for cell culture experiments

Chemicals	Company
AEC substrate kit	Vector Laboratories, Burlingame, USA
BSA (bovine serum albumin)	Roth, Karlsruhe, Germany
DAKO Target Retrieval Solution pH 6 (10x concentrated)	Agilent, Santa Clara, California, USA
DMEM (Dulbecco´s modified eagle medium) 1 g/l glucose; without phenol red	ThermoFisher Scientific, Waltham, Massachusetts, USA
DMSO (dimethyl sulfoxide)	Sigma-Aldrich, Taufkirchen, Germany
Eosin	Roth, Karlsruhe, Germany
FCS (fetal calf serum)	Sigma-Aldrich, Taufkirchen, Germany
Hematoxylin	Roth, Karlsruhe, Germany
HEPES	PAA Laboratories, Cölbe, Germany
PBS (phosphate buffered saline)	PAA Laboratories, Cölbe, Germany
Penicillin/Streptomycin	PAA Laboratories, Cölbe, Germany

Table 3.4
Media and solutions used for experiments

Media and solutions	Composition
Cell culture medium	DMEM with 10% heat-inactivated FCS 100 U/ml penicillin 10 µg/ml streptomycin 10 mM HEPES storage at 4 °C
Freezing medium	FCS with 10% DMSO storage at 4 °C

Table 3.5
Enzymes, receptor agonists and antibodies

Enzymes, Proteins and Antibodies	Company
Accutase	PAA Laboratories, Cölbe, Germany
Collagenase	Sigma-Aldrich, Taufkirchen, Germany
Dispase II	PAN, Aidenbach
Fenoldopam hydrochloride	Tocris Bioscience, Bristol, United Kingdom
Fibronectin	Sigma-Aldrich, Taufkirchen
Histofine Simple Stain MAX PO (multi) antimouse, antirabbit	Nichirei Biosciences, Tokyo, Japan
Ropinirole	Tocris Bioscience, Bristol, United Kingdom
Trypsin/EDTA	PAA Laboratories, Cölbe, Germany

Table 3.6
Antibodies used for immunohistochemistry on human tissues

Antibodies	**Company**
anti-D1DR (monoclonal mouse IgG2b)	Santa Cruz Biotechnology, Dallas, Texas, USA
anti-D2DR (polyclonal rabbit IgG)	Acris/OriGene, Herford, Germany
anti-D3DR (monoclonal mouse IgG2a)	Biolegend San Diego, California, USA
anti-D4DR (polyclonal rabbit IgG)	OriGene; Herford, Deutschland
anti-D5DR (monoclonal mouse IgG1)	Santa Cruz Biotechnology; Dallas, Texas, USA
normal monoclonal mouse-IgG2b	Santa Cruz Biotechnology; Dallas, Texas, USA
normal polyclonal rabbit-IgG	Agilent; Santa Clara, California, USA
normal polyclonal rabbit-IgG	Biolegend; San Diego, California, USA
normal monoclonal mouse-IgG1	Santa Cruz Biotechnology; Dallas, Texas, USA

Table 3.7
Kits used for ELISA experiments

Kits	**Company**
Human IL-6 Quantikine ELISA kit	R&D Systems, Wiesbaden, Germany
Human IL-8 Quantikine ELISA kit	R&D Systems, Wiesbaden, Germany
Human MMP-3 Quantikine ELISA kit	R&D Systems, Wiesbaden, Germany
Human pro-MMP-1 Quantikine ELISA kit	R&D Systems, Wiesbaden, Germany

Table 3.8
Equipment used for experiments

Equipment	Company
BioPhotometer	Eppendorf, Hamburg, Germany
Centrifuge 5471C	Eppendorf, Hamburg, Germany
Cytospin II	Shandon Lipshaw Inc., Pennsylvania, USA
LC Carousel Centrifuge	Roche Diagnostics, Mannheim, Germany
Leica DC 200 (Camera)	Leica Microsystems, Wetzlar, Germany
Leica DM IRB (Microscope)	Leica Microsystems, Wetzlar, Germany
Sunrise ELISA Reader	Tecan, Crailsheim, Germany
Vortex Genie 2	Bender & Hobein AG, Zürich, Switzerland
Leitz 1516 (rotary microtome)	Leica Microsystems, Nussloch, Germany
Steam cooker	Russell Hobbs, Sulzbach, Germany

Table 3.9
Software used for experiments

Software	Company
GraphPad Prism 5	GraphPad Software, La Jolla, California, USA
IM 1000	Leica Microsystems, Wetzlar, Germany
LightCycler Software 3.5	Roche Diagnostics, Mannheim, Germany
Magellan 5	Tecan, Crailsheim, Germany
ImageJ	Open Source
Microsoft Powerpoint	Microsoft Corporation, Redmond, Washington, USA

3.3 Methods

3.3.1 Cell culture

Extraction of synovial fibroblasts

After surgery, the tissue was directly taken to the laboratory for processing. Whereas some of the tissue was embedded in paraffine for later immunohistochemical and hematoxylin and eosin stainings, the other parts of the synovium were used for isolation of the synovial fibroblasts.
The isolation process was conducted according to the standard operating protocol of the laboratory.[255,256] In order to increase the number of synovial fibroblasts for later experiments, but also to eradicate other cell types like macrophages, the cells were cultured up to passage 4-5.

Freezing and storage of synovial fibroblasts

Synovial fibroblasts were cultured until they reached 90% confluence on the plate. The cells were detached by using trypsin (5 g/l) and then centrifuged afterwards for 10 minutes by 300 x g.
Then, the SF were resuspended in 4ml of freezing medium (Tbl 3.4) for each cryovial, immediately put on ice and quickly stored at -80°C. After 24 hours, the cells were transferred into liquid nitrogen for long term storage.

Thawing of synovial fibroblasts

Before thawing, the cryovials with SF were taken out of the liquid nitrogen tank and put on ice to avoid immediate thawing. In order to use SF for cell culture, the vials were thawed in a 37° C water bath and diluted in 10ml of prewarmed cell culture medium (Tbl 3.4) directly afterwards.
Cells were centrifuged for 10 minutes by 300 x g. After removing the supernatant, the cells were diluted in prewarmed cell culture medium and equally

distributed in 75 ccm cell culture flasks. In order to remove all of the remaining DMSO and also dead cells, the cell culture medium was changed the next day.

Passaging of synovial fibroblasts

Synovial fibroblasts were passaged in a 1:2 relation after reaching about 80-90% confluence. After removing the supernatant medium, cells were briefly washed with PBS. Detachment of the cells was achieved by using 3ml trypsin (5g/l) followed by incubation at 37° C for 3-4 minutes.
This was followed by centrifugation for 10 min at 300 x g and subsequent dilution with cell culture medium and equal distribution of the cells in 75 ccm cell culture flasks. For the experiments, only cells in passage 4-5 were used.

3.3.2 Immunohistochemistry

To evaluate the distribution of dopamine receptors in the synovium, particularly in the invasion zone, paraffin-embedded tissue samples were used to perform immunohistochemical staining (available at the tissue bank of the research facility) for D1DR, D2DR, D3DR and D5DR. D4DR-stainings were performed in the laboratory of Silvia Capellino at the Leibniz Institute in Dortmund.
All four dopamine receptor subtypes were stained by using specific antibodies for each receptor subtype, respectively (Tbl. 3.6). The tissue was cut into 5µm sections and transferred on microscope slides. The slides were pre-incubated at 60℃ for 45 minutes to facilitate the removal of paraffin. The remaining paraffin was then extracted using Xylene and Ethanol baths in decreasing concentrations (100% ethanol followed by 96%, 80%, 70%, 50% ethanol, 5 minutes each).
For the following antigen retrieval, the tissue was incubated at 90℃ in DAKO citrate buffer for 20 minutes. Unspecific binding sites were blocked by an 8% peroxide solution followed by a blocking solution containing 10% each of BSA, chicken and fetal calf serum.
Incubation with the first antibody for respective DR was performed in a moist chamber at 4℃ over night, followed by an incubation step with the second antibody (Histofine) for 30 minutes at room temperature. Using AEC substrate, color development was controlled under the microscope. In addition, isotype controls were performed for all receptor subtypes.

3.3.3 Migration Assay

The influence of RASF and OASF migration by DR activation was further investigated by using a two-chamber migration assay (Boyden Chamber).
Here, specific D1-like or D2-like receptor agonists were added to the medium in 10^{-6} M and 10^{-7} M, respectively, and additionally 10^{-8} M for the D2-like receptor agonist. For D1-like receptor activation, fenoldopam hydrochloride was used, and for D2-like receptor activation ropinirole. As both D1-like and D2-like receptor agonists were water soluble, they were directly dissolved in DMEM medium.

In order to promote migration of SF, FCS in culture media (Tbl. 3.4) was reduced from 10% to 2% for 6 hours prior to the experiment. Basic principle of this experiment is to separate the upper chamber filled with SF and only 2% FCS from the bottom chamber filled with 10 % FCS by a porous membrane. In order to get to the more nutritious medium, SF have to actively migrate through the pores. In this setting, a membrane with pores of 8µm was used.
DR agonists were added to the medium of both the upper and bottom chamber. During preparations for the experiment, SF were detached from the flasks by using accutase and counted using a Neubauer chamber after starving them for 6 hours.
For each upper chamber, 30,000 cells were diluted in 50µl medium containing 2% FCS and the specific receptor agonist in respective concentrations. 6 wells per stimulation were prepared. The bottom chamber was filled with 30µl medium and the respective receptor agonist. The migration chamber was placed into the incubator at 37℃ for 16 hours.
Afterwards, SF adherent to the bottom side of the membrane were fixed with cold methanol (4℃), stained with hematoxylin and counted afterwards (Fig. 3.1). In order to count the migrated cells, three areas of the well were photographed in 10-fold magnification and afterwards counted by using the ImageJ software.

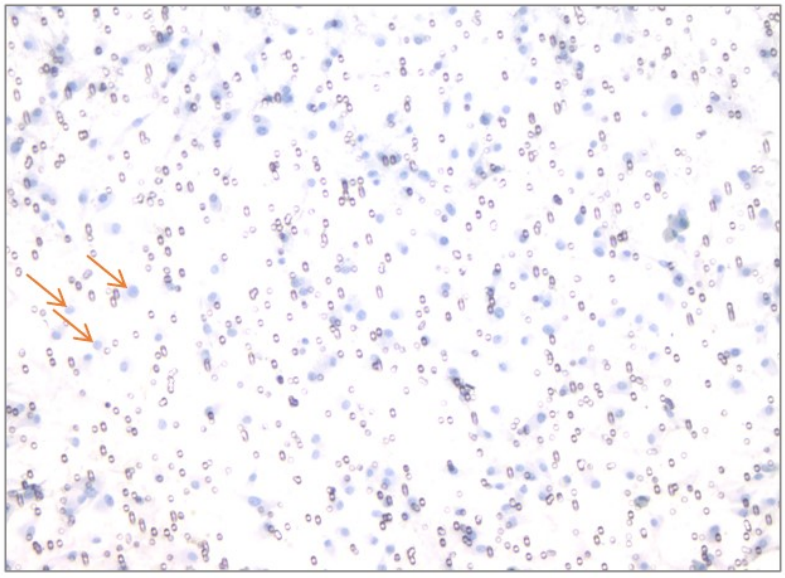


Figure 3.1
Representative picture of a membrane with previously fixated and HE-stained SF (marked with orange arrows). Three representative pictures were taken per well, counted and afterwards compared to an unstimulated control. Photos were taken in 10-fold magnification.

3.3.4 Wound Healing Assay

To further investigate the influence of dopamine receptor activation on cell motility of SF, a wound healing assay was performed.
SF were cultured up to passage 4 with DMEM and then equally distributed on 48-well plates. Here, the outer wells of the plates were left blank and only filled with PBS in order to prevent the inner wells from drying out. SF were further cultivated until they reached 95-100% confluence.
For scraping off the cells in the middle of the wells, a 100µl pipet tip was used. After a quick rinse with PBS in order to remove loose cells, respective stimulants in cell culture medium were added to the wells. For control, DMEM cell culture medium with 10 % FCS was used. The other wells were treated with specific D1-like or D2-like receptor agonists in concentrations of 10^{-6} M and 10^{-7} M respectively and additionally 10^{-8} M of D2-like receptor agonists diluted in DMEM medium containing 10% FCS.
For D1-like receptor activation, fenoldopam hydrochloride was used, and for

D2-like receptor activation ropinirole. As both D1-like and D2-like receptor agonists were water soluble, they were directly dissolved in DMEM medium. In order to evaluate cell motility over time, two pictures per well were taken directly after scraping (0 hours) and 10, 12, 14 and 16 hours after scraping (Fig. 3.2). Pictures in 10-fold magnification were used for evaluation.
Cells which had moved into the gap at the documented time points were counted and compared to the unstimulated control wells at the same time.

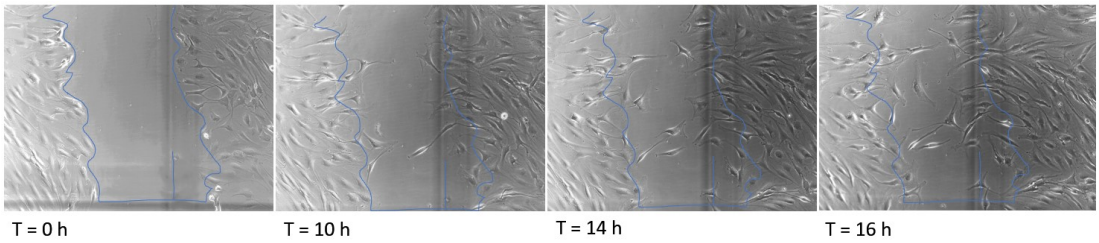


Figure 3.2
Representative picture of Scrape assay at different time points. RASF and OASF were cultured in 48-well plates until they reached 95-100% confluence. For scraping off the cells in the middle of the wells, a 100µl pipet tip was used. Pictures were taken directly after scraping (T=0 h) and after 10 h (T=10 h), 12 h (not shown above), 14 h (T=14 h) and 16 h (T=16 h).
Cells that moved into the gap were counted and compared to the unstimulated control at the same time point. Photos were taken in 10-fold magnification.

3.3.5 ELISA

Enzyme Linked Immunosorbent Assays (ELISA) for IL6, MMP3 and pro-MMP1 were performed in order to determine both inflammatory cytokines and matrix degrading enzymes under specific D1-like and D2-like receptor activation.
Here, D1-like or D2-like receptor agonists in 10^{-6} M, and 10^{-7} M respectively, and additionally 10^{-8} M of D2-like receptor agonists were used. For D1-like receptor activation, fenoldopam hydrochloride was used, and for D2-like receptor activation ropinirole. The experiment was performed in 24-well plates with RASF and OASF in passage 5.
SF were cultured for 24 hours under respective stimulation before supernatants were collected and immediately stored at - 20 ℃ until use.
ELISAs were performed with commercially available kits according to the manufacturer's instructions and analyzed by using the Magellan 5 software.

3.3.6 Statistical analysis

Data and statistical analysis of the experiments was performed by using Microsoft Excel and GraphPad Prism 8 for Microsoft Windows. Friedman test and Dunn's multiple comparison test and for correlation analysis, Spearman non-parametric correlation was performed. Results were considered significant when $p \leqq 0.05$.

Chapter 4

Results

4.1 DR are present in the invasion zone and synovium of RA and OA patients

To investigate the distribution of DR within the synovium, we performed immunohistochemical staining for four of the five dopamine receptor subtypes in the synovium of RA and OA patients and in the invasion zone of RA patients. The area where synovial cells aggressively invade the joint cartilage is referred to as the invasion zone (Fig. 1.6). Our objective was to determine whether DR expression is altered in or near the invasion zone or the lining layer of the synovium in compared to other regions of the synovium.

In all investigated samples, all four receptor subtypes were expressed, in both RA and OA patients. All DR subtypes were stronger expressed in the lining than in the sublining layer in both RA and OA patients. Of note, D2DR was stronger expressed in RA than in OA synovium (Fig. 4.2).
Overall, D3DR seemed to be equally expressed in RA and OA synovium with higher expression in the lining layer compared to the sublining layer (Fig. 4.3). D1DR and also D5DR staining was only slightly stronger in RA than in OA synovium (Fig. 4.1 and Fig. 4.4). Representative pictures of the DR stainings are shown in Fig. 4.1 - Fig. 4.4.

All four DR were also present in the invasion zone, although only D1DR, D2DR and D5DR signals were stronger in the invasion zone compared to the sublining synovial tissue area. D3DR was present in the invasion zone, too but the signal level was not altered compared to the sublining tissue (Fig. 4.5).

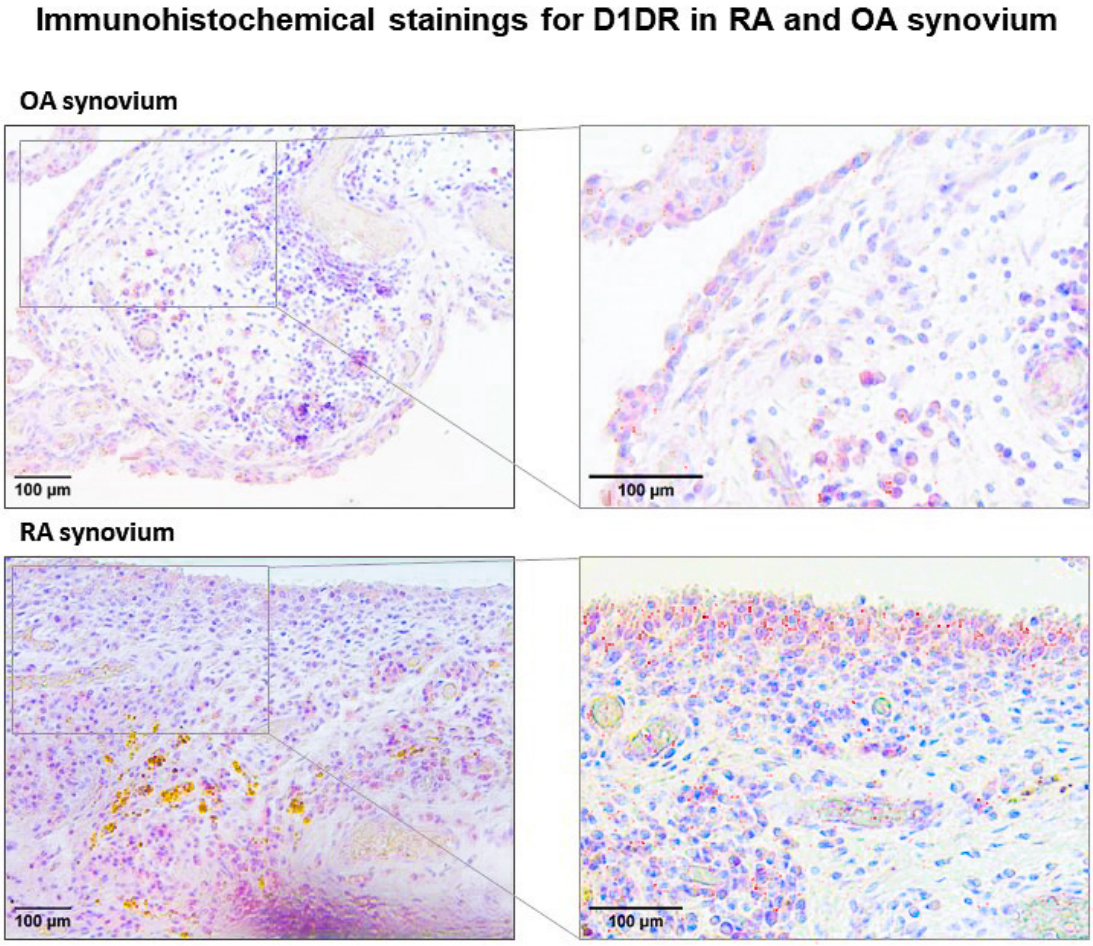


Figure 4.1
Immunohistochemical staining of D1DR (red staining) in synovial tissue of OA and RA patients. D1DR were present in both RA and OA synovium increased expression in the lining layer compared to the sublining layer in both RA and OA synovium. Staining intensity of D1DR seemed to be slightly weaker in OA than in RA. Representative photos of RA and OA synovium are presented here. SF were also stained with hematoxylin (blue) for better tissue overview. SL = sublining layer; LL = lining layer. 20-fold magnification on the left column with magnified view of representative areas on the right.

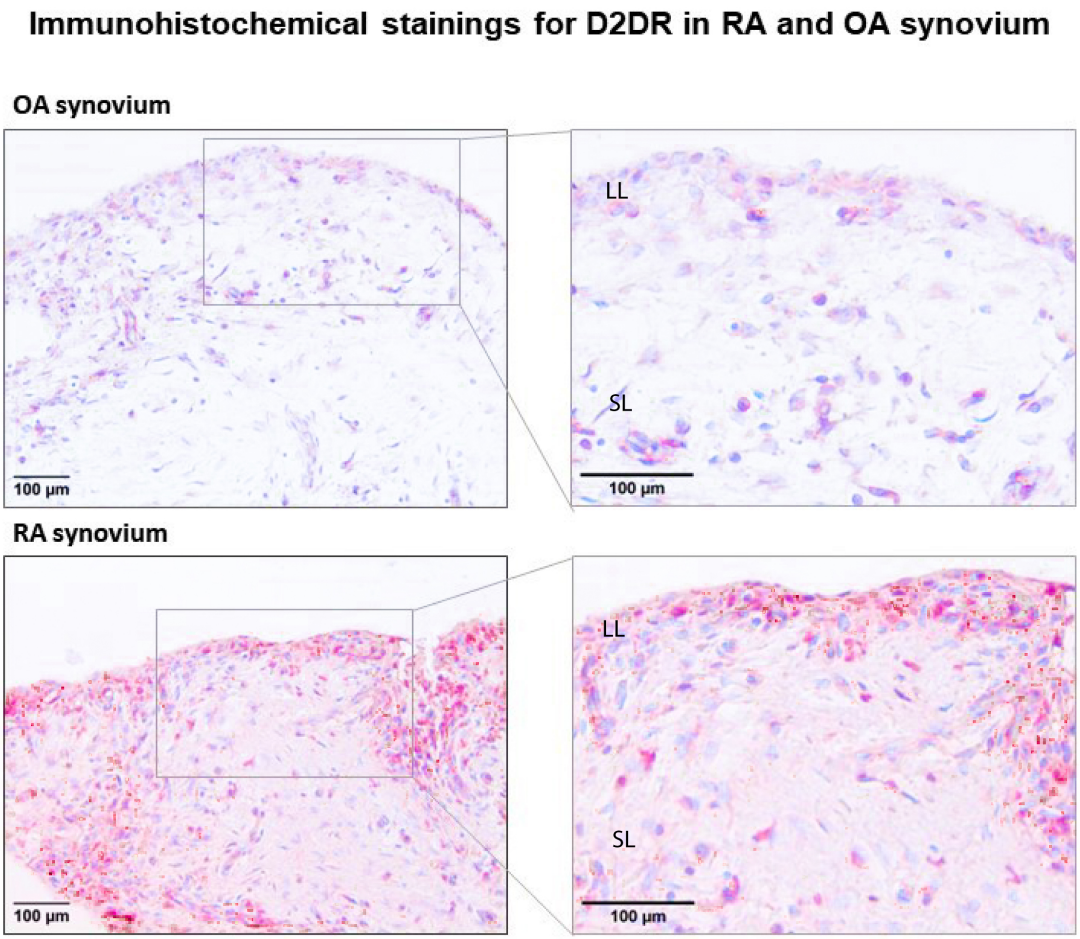


Figure 4.2
Immunohistochemical staining of D2DR (red staining) in synovial tissue of OA and RA patients. D2DR were present in both RA and OA synovium and stronger expressed in the lining layer than in the sublining layer in both Ra and OA synovium. Staining intensity of D2DR was stronger in RA than in OA.
Representative photos of RA and OA synovium are presented here. SF were also stained with hematoxylin (blue) for better tissue overview. SL = sublining layer; LL = lining layer. 20-fold magnification on the left column with magnified view of representative areas on the right.

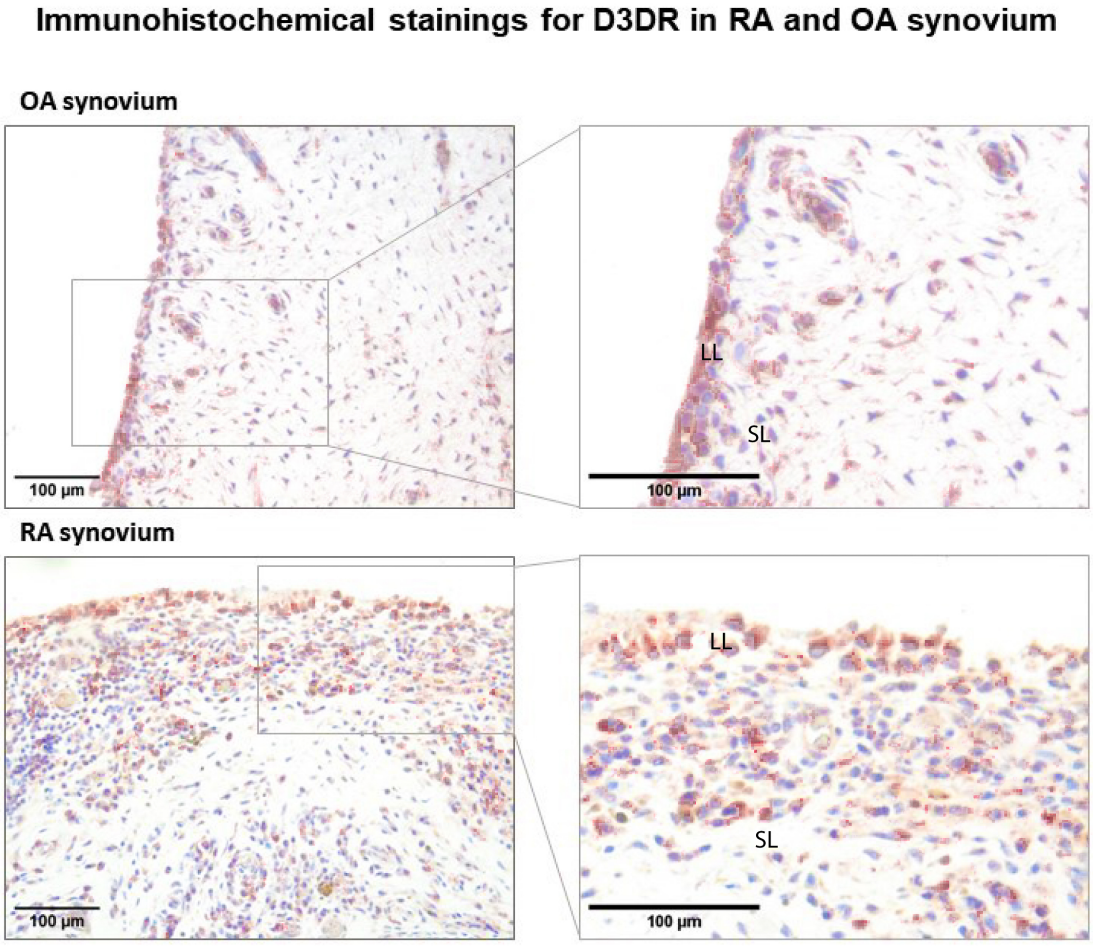


Figure 4.3
Immunohistochemical staining of D3DR (red staining) in synovial tissue of OA and RA patients. D3DR were present in both RA and OA synovium and stronger expressed in the lining layer than in the sublining layer in both RA and OA synovium. Staining intensity of D3DR was not altered between RA and OA synovium. Representative photos of RA and OA synovium are presented here. SF were also stained with hematoxylin (blue) for better tissue overview. SL = sublining layer; LL = lining layer. 20-fold magnification on the left column with magnified view of representative areas on the right.

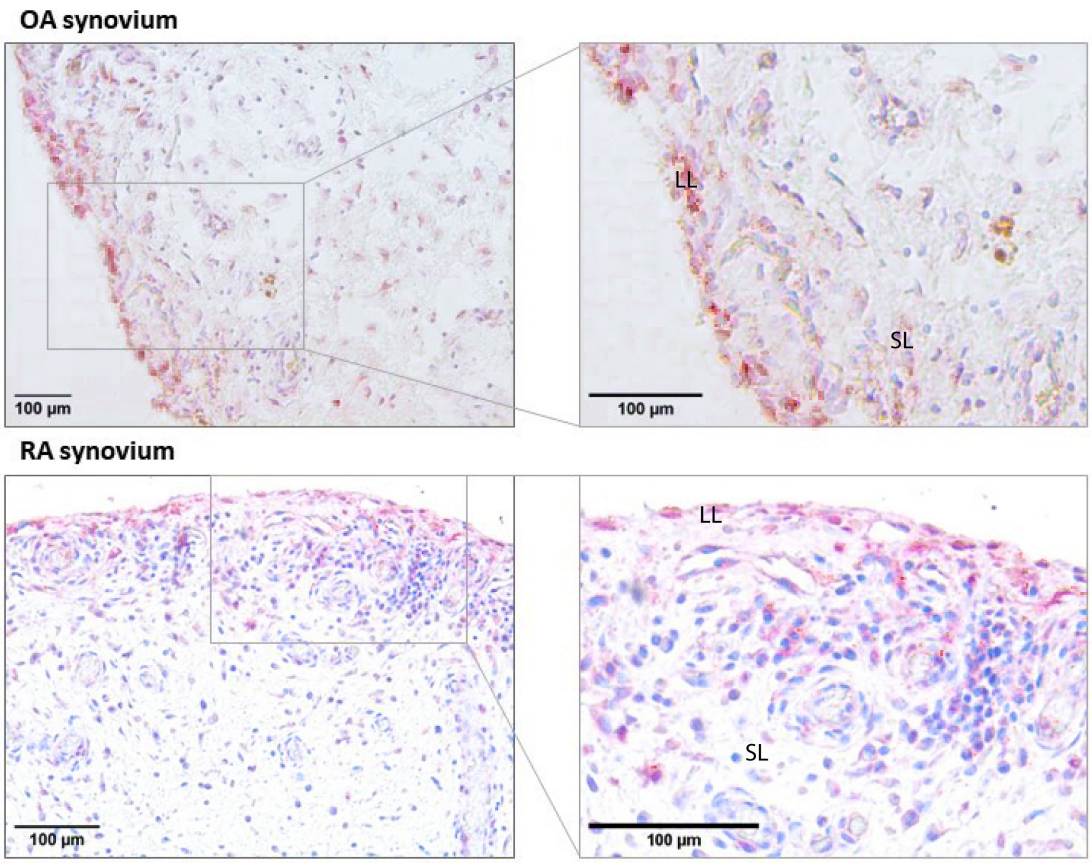


Figure 4.4
Immunohistochemical staining of D5DR (red staining) in synovial tissue of OA and RA patients. D5DR were present in both RA and OA synovium and stronger expressed in the lining layer than in the sublining layer of RA and OA synovium. Staining intensity of D5DR tended to be stronger in RA than in OA. Representative photos of RA and OA synovium are presented here. SF were also stained with hematoxylin (blue) for better tissue overview. SL = sublining layer; LL = lining layer. 20-fold magnification on the left column with magnified view of representative areas on the right.

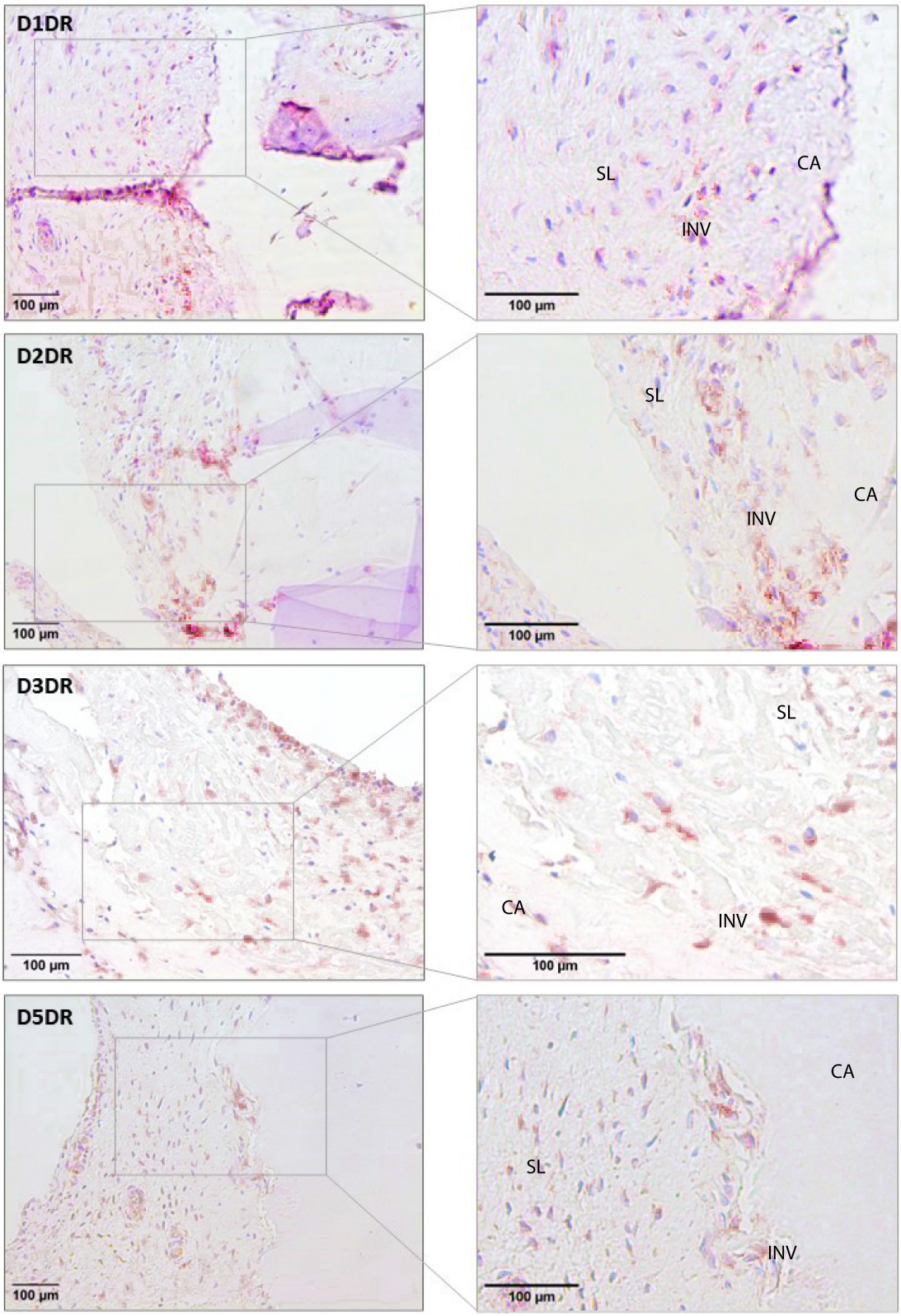


Figure 4.5
Immunohistochemical stainings of D1DR, D2DR, D3DR and D5DR (red staining) in the invasion zone of RA patients. All four DR are present in the invasion zone. D1DR, D2DR and D5DR were stronger expressed in the invasion zone. D3DR expression was not altered in the INV compared to the sublining area. RASF were also stained with hematoxylin (blue) for better tissue overview. SL = sublining layer; INV = invasion zone; CA = cartilage. 20-fold magnification on the left column with magnified view of representative areas on the right.

4.2 Migration of SF under DR activation is age-dependent in RA and OA

As the immunohistochemical stainings showed an increased signal of DR in the invasion zone, the next step was to investigate whether specific D1-like or D2-like dopamine receptor activation in different concentrations affects cell migration. Therefore, we performed a migration assay using a Boyden Chamber with SF obtained from RA and OA patients.

When comparing the migration of RASF and OASF under respective DR activation with an unstimulated control, no significant changes could be observed due to vast scattering of migrated cells (Tbl. 4.2a and Tbl. 4.2b). No differences were observed between RA and OA patients and migration of RASF did not correlate with disease duration of RA (Tbl. 4.1). However, migration of RASF and also OASF after dopamine receptor activation highly correlates with the age of the patients at surgery (Fig. 4.6 and Fig. 4.7). Synovial fibroblasts obtained from OA patients showed a significant age-dependent change in migration after both D1-like and D2-like receptor activation. In "younger" patients (under 75 years old), both receptor agonists in a concentration of 10^{-6} M led to an increase of cell migration up to 50%. In older patients, the same receptor activation led to a significant reduction of migration up to 50%. No differences could be observed between D1-like and D2-like receptor activation.
The strongest age-dependent effects could be observed with fenoldopam and ropinirole in 10^{-6}M concentration. Lower concentrations of D1-like and D2-like receptor agonists (fenoldopam in 10^{-7}M and ropinirole 10^{-7}M and 10^{-8}M) resulted in the same age-dependent effect, but to a lower extent (Fig. 4.6 and 4.7). A significant inverse age-dependent correlation was shown for OASF treated with fenoldopam in 10^{-6} M (n=9; p=0.0031; r=-0.8833) and also 10^{-7} M (n=8; p=0.0046; r=-0.9048) and ropinirole with 10^{-6} M (n=9; p=0.0020; r=-0.9000). Other tested concentrations of ropinirole with 10^{-7} M (n=9; p=0.4933; r=0.1323) and 10^{-8} M (n=8; p=0.1323; r=-0.5952) were also age-dependent but not significant (Fig. 4.6 and Fig. 4.7).

Migration of RASF was similar to OASF. Here, a significant age-dependent inverse correlation was also shown. RASF of younger patients reacted with a significant increase of migration up to 150% under ropinirole and 70% under fenoldopam, whereas in older patients cell migration was inhibited up to 50% by both receptor agonists. Overall, the induced migration of RASF was higher compared to OASF.

Although an age-dependent inverse correlation was found in all used concentrations in RASF, only fenoldopam at 10^{-6} M (n= 9; p=0.0214; r=-0.7667) and ropinirole at 10^{-6} M (n=9; p=0.0172; r=-0.7833) and 10^{-8} M (n=9; p=0.0045; r=-0.8667) were significant. Fenoldopam and ropinirole in 10^{-7} M did also not result in an age-dependent effect in RASF (fenoldopam 10^{-7} M: n=9; p=0.2696; r=-0.4167; ropinirole 10^{-7} M: n=9; p=0.0666; r=-0.6500; Fig. 4.6 and 4.7).

Of interest, baseline migration of both RASF and OASF without any receptor agonist was not age-dependent (data not shown).

Table 4.1

Correlation between disease duration and migration of RASF under D1-like and D2-like DR activation.

We observed no correlation between the known duration of RA and cell migration under both D1-like and D2-like receptor agonists.

For migration assessment, a Boyden Chamber was used. Migrated cells of RASF were counted and compared to RASF without the respective agonist (control). The difference was expressed in percent and then correlated with the known duration of RA. P-values $\leqslant 0.05$ were considered as significant.

Treatment	n	mean	SD	significance
Fenoldopam 10^{-6} M	9	-0.2000	0.6134	no
Fenoldopam 10^{-7} M	9	-0.1500	0.7081	no
Ropinirole 10^{-6} M	9	-0.0500	0.9116	no
Ropinirole 10^{-7} M	8	0.1190	0.7930	no
Ropinirole 10^{-8} M	8	-0.1905	0.6646	no

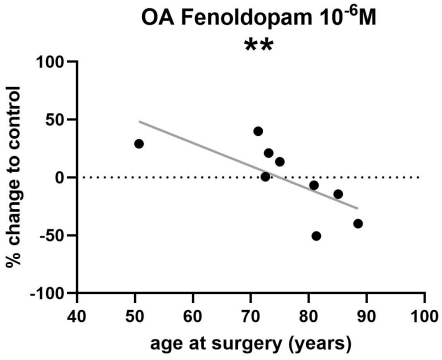
Table 4.2
Migration of OASF and RASF under D1-like and D2-like DR activation vs. unstimulated control.
Due to vast scattering, no significant changes in migration were observed under both D1-like and D2-like receptor activation compared to the unstimulated control.
For migration assessment, a Boyden Chamber was used. SF were stimulated with different concentrations of D1-like (fenoldopam 10^{-6} and 10^{-7} M) and D2-like (ropinirole 10^{-6} - 10^{-8} M) receptor agonists. Migrated cells of OASF and RASF were counted and compared to SF without the respective agonist (control).
P-values $\leqslant$ 0.05 were considered as significant.

Treatment	**n**	**mean**	**SD**	**significance**
Control	9	169.1	107.1	
Fenoldopam 10^{-6} M	9	189.3	140.7	no
Fenoldopam 10^{-7} M	9	187.2	155.2	no
Ropinirole 10^{-6} M	9	208.7	149.5	no
Ropinirole 10^{-7} M	9	173.3	137.9	no
Ropinirole 10^{-8} M	9	172.4	113.6	no

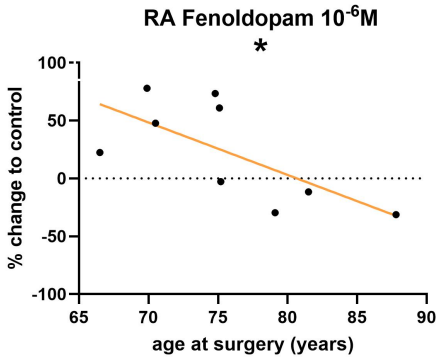
(a) Migration of OASF under D1-like and D2-like DR activation vs. unstimulated control

Treatment	**n**	**mean**	**SD**	**significance**
Control	10	217.3	128.3	
Fenoldopam 10^{-6} M	10	249.4	166.1	no
Fenoldopam 10^{-7} M	10	211.7	110.9	no
Ropinirole 10^{-6} M	10	232.6	99.5	no
Ropinirole 10^{-7} M	10	220.0	124.1	no
Ropinirole 10^{-8} M	10	224.2	125.3	no

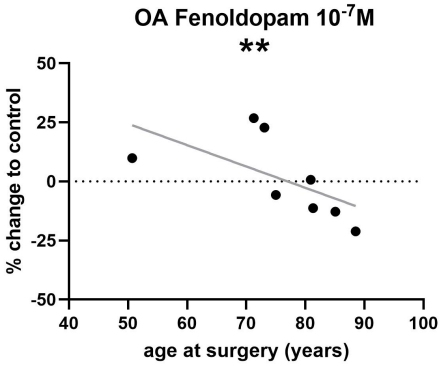
(b) Migration of RASF under D1-like and D2-like DR activation vs. unstimulated control



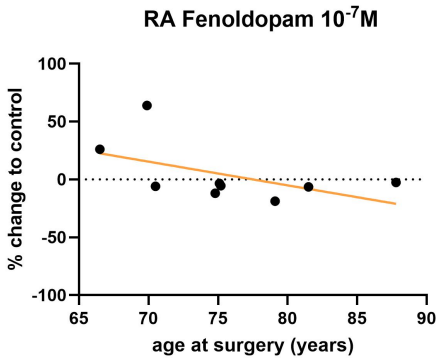
(a) Age-dependent migration of OASF under D1-like DR activation with fenoldopam 10^{-6}M



(b) Age-dependent migration of RASF under D1-like DR activation with fenoldopam10^{-6}M

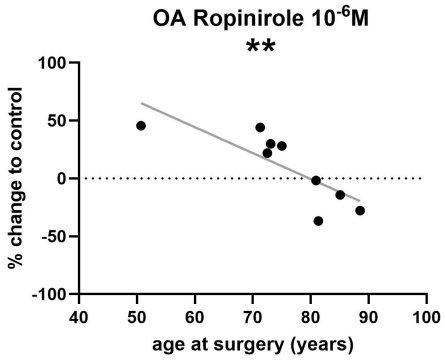


(c) Age-dependent migration of OASF under D1-like DR activation with fenoldopam 10^{-7}M

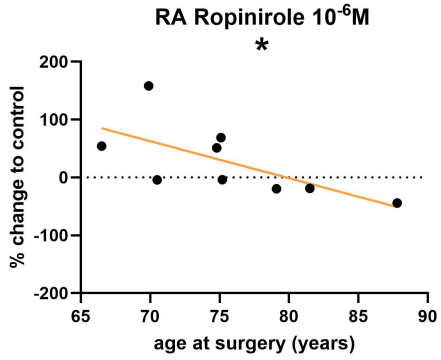


(d) Age-dependent migration of RASF under D1-like DR activation with fenoldopam 10^{-7}M

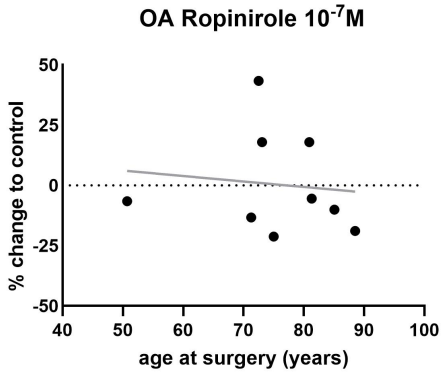
Figure 4.6
Significant age-dependent migration of OASF and RASF (a, b) under D1-like receptor activation with fenoldopam 10^{-6}M (OASF n=9; p=0.0031; r=-0.8833 and RASF n=9; p=0.0214; r=-0,7667) and OASF (c) with fenoldopam 10^{-7}M (n=8; p=0.0046; r=-0.9048). Migration of RASF under fenoldopam 10^{-7}M (n=8; p=0.2696; r=-0.4167) was not significant (d). For migration assessment, a Boyden Chamber was used. Cells were stimulated with a specific D1-like receptor agonist (fenoldopam) in different concentrations (10^{-6}M and 10^{-7}M). Migrated cells of OASF and RASF were counted and compared to OASF and RASF without the respective agonist (control). The difference between unstimulated control vs. stimulated was expressed in percent and then correlated with the age of the patients at surgery. P-values $\leqslant 0.05$ were considered as significant.



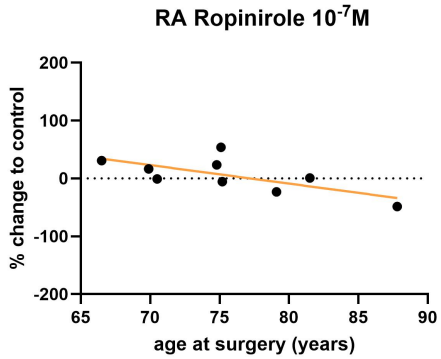
(a) Age-dependent migration of OASF under D2-like DR activation with ropinirole 10^{-6}M



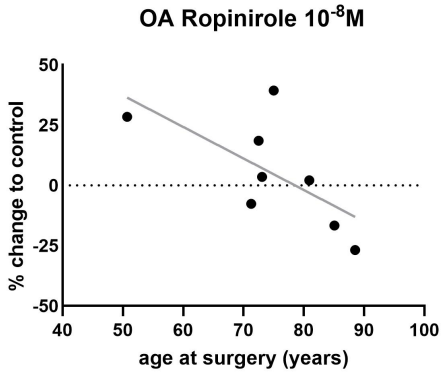
(b) Age-dependent migration of RASF under D2-like DR activation with ropinirole 10^{-6}M



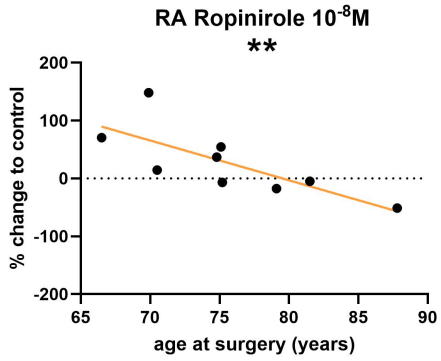
(c) Age-dependent migration of OASF under D2-like DR activation with ropinirole 10^{-7}M



(d) Age-dependent migration of RASF under D2-like DR activation with ropinirole 10^{-7}M



(e) Age-dependent migration of OASF under D2-like DR activation with ropinirole 10^{-8}M



(f) Age-dependent migration of RASF under D2-like DR activation with ropinirole 10^{-8}M

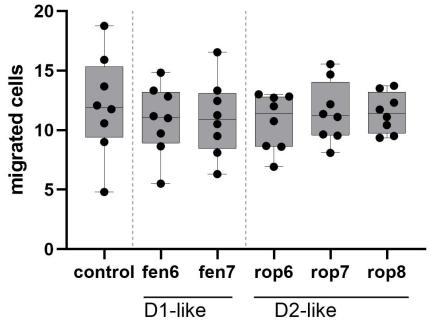
Figure 4.7
Significant age-dependent migration of both OASF and RASF (Fig. a, b) under D2-like receptor activation with ropinirole 10^{-6}M (OASF: n=9; p=0.0020, r=-0.9000 and RASF: n=9; p=0.0172; r=-0.7833). Migration of RASF treated with ropinirole 10^{-8}M (Fig. f, RASF n=9;p=0.0045; r=-0.8667) was also significantly age-dependent. Both RASF and OASF treated with ropinirole 10^{-7}M (Fig. c, d) showed only low age-dependent migration, and not to a significant extent (RASF: n=9;p=0.0666; r=-0.6500 and OASF: n=9; p=0.4933; r=-0.2667). Same was true for OASF under 10^{-8}M ropinirole treatment (Fig. e, n=9; p=0.1323; r=-0.5952). The experimental design matches the one described in Fig. 4.6

4.3 Cell motility of both RASF and OASF under DR activation is also age-dependent.

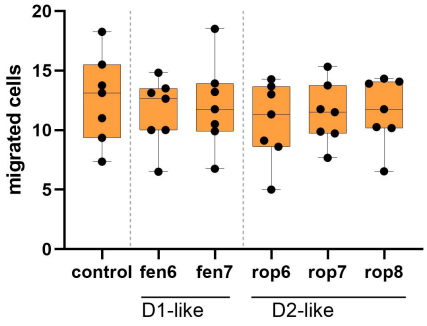
In contrast to the Boyden chamber migration assay, migration of SF in the wound healing assay is not directed towards a gradient but shows increased cell motility. As cell motility is an important trait of fibroblasts and might also be affected by DR activation, we performed a scrape assay with specific D1-like (fenoldopam) and D2-like (ropinirole) receptor agonists.

Similar to the Boyden assay, we performed the experiments with different concentrations of the agonists. Also, cells were observed and counted 10, 12, 14 and 16 hours after the beginning of the experiments.
Comparing cell motility of OASF and RASF under respective DR activation with the unstimulated control, no significant changes could be observed (Fig. 4.8 and Tbl. 4.3 - 4.10). In the control group of OASF (n=7), 14.80 $\pm$ 5.50 cells migrated on average ($\pm$SD) into the gap. After D1-like and D2-like receptor activation, no significant differences could be observed (fenoldopam (mean $\pm$ SD): 14.18 $\pm$3.48 cells migrated at 10^{-6} M and 14.15 $\pm$ 4.02 cells migrated at 10^{-7} M; ropinirole (mean $\pm$ SD): 13.53 $\pm$ 2.21cells migrated under 10^{-6} M, 13.62$\pm$ 2.40 cells migrated under 10^{-7} M and 14.08 $\pm$ 1.93 cells migrated under 10^{-8} M. p >0.05).
Similar results were observed in cell motility assays performed with SF from RA patients (n=7): on average ($\pm$ SD) 16.04 $\pm$ 3.78 cells migrated in the unstimulated control group, which did not significantly change under D1- like or D2-like dopamine receptor activation (fenoldopam (mean $\pm$ SD): 14.90 $\pm$ 2.71 cells migrated at 10^{-6} M and 15.12 $\pm$ 4.13 cells migrated at 10^{-7} M; ropinirole (mean $\pm$ SD): 13.98 $\pm$ 3.73 migrated at 10^{-6} M, 14.59 $\pm$ 3.18 cells migrated at 10^{-7} M and 14.53 $\pm$ 3.04 cells migrated at 10^{-8} M. p >0.05).

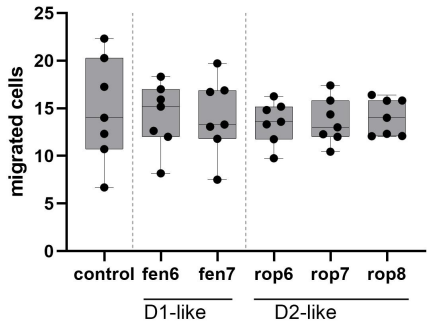
Taken together, results from these assays are similar to the ones obtained from the Boyden chamber migration assay, although overall effects of DR agonists on SF were lower compared to the results from the migration assay.



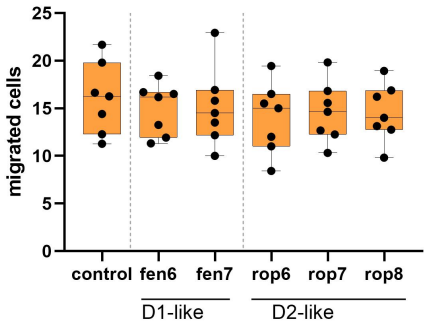
(a) Migrated OASF (n=8) after 10 hours.



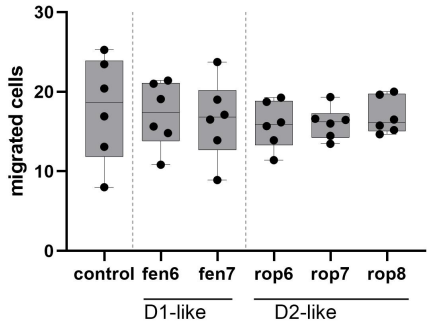
(b) Migrated RASF (n=7) after 10 hours



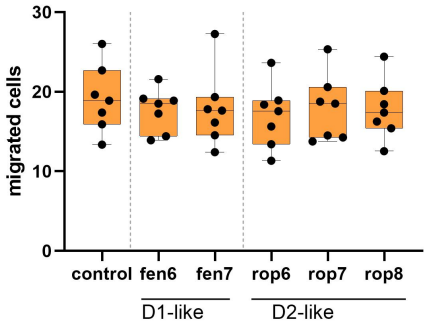
(c) Migrated OASF (n=7) after 12 hours



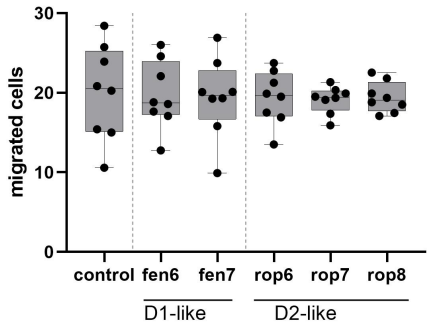
(d) Migrated RASF (n=7) after 12 hours



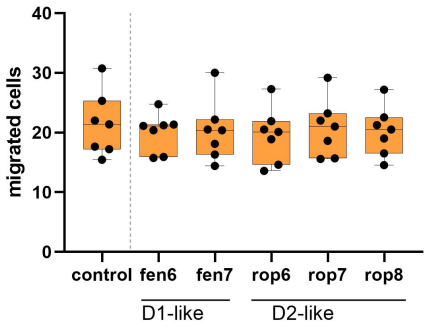
(e) Migrated OASF (n=6) after 14 hours



(f) Migrated RASF (n=7) after 14 hours



(g) Migrated OASF (n=8) after 16 hours



(h) Migrated RASF (n=7) after 16 hours

Figure 4.8
Cell motility of OASF (left column; a, c, e) and RASF (right column; b, d, f) under D1-like receptor activation (fenoldopam in 10^{-6} M and 10^{-7} M) and D2-like receptor activation (ropinirole in 10^{-6} M - 10^{-8} M). For motility assessment, a wound healing assay (scrape assay) was used. Cells migrated into the scrape were counted 10, 12, 14 and 16 hours after stimulation. No significant changes between respective receptor activation and the unstimulated control could be observed in OASF or RASF.

Table 4.3
Cell motility of RASF under D1-like receptor activation using fenoldopam in 10^{-6} M and 10^{-7} M and D2-like receptor activation using ropinirole in 10^{-6} M - 10^{-8} M after 10 hours. For motility assessment, a wound healing assay (scrape assay) was used. Cells migrated into the scrape were counted 10, 12, 14 and 16 hours after stimulation. No significant changes between respective receptor activation and the unstimulated control could be observed. The corresponding graph was shown before(Fig. 4.8b).

Treatment	**Migrated cells** (mean)	**SD**	**n**	**p-value**	**significance**
Control	12.62	±3.705	7		
Fenoldopam 10^{-6} M	11.51	±2.844	7	>0.9999	no
Fenoldopam 10^{-7} M	12.08	± 3.687	7	>0.9999	no
Ropinirole 10^{-6} M	10.71	±3.333	7	0.0921	no
Ropinirole 10^{-7} M	11.37	±2.588	7	>0.9999	no
Ropinirole 10^{-8} M	11.58	±2.839	7	>0.9999	no

Table 4.4
Cell motility of RASF under D1-like receptor activation using fenoldopam in 10^{-6} M and 10^{-7} M and D2-like receptor activation using ropinirole in 10^{-6} M - 10^{-8} M after 12 hours. For motility assessment, a wound healing assay (scrape assay) was used. Cells migrated into the scrape were counted 10, 12, 14 and 16 hours after stimulation. No significant changes between respective receptor activation and the unstimulated control could be observed. The corresponding graph was shown before (Fig. 4.8d).

Treatment	**Migrated cells** (mean)	**SD**	**n**	**p-value**	**significance**
Control	16.04	±3.781	7		
Fenoldopam 10^{-6} M	14.90	±2.713	7	>0.9999	no
Fenoldopam 10^{-7} M	15.12	±4.130	7	>0.9999	no
Ropinirole 10^{-6} M	13.98	±3.729	7	0.1606	no
Ropinirole 10^{-7} M	14.59	±3.182	7	>0.9999	no
Ropinirole 10^{-8} M	14.53	±3.036	7	0.9927	no

Table 4.5
Cell motility of RASF under D1-like receptor activation using fenoldopam in 10^{-6} M and 10^{-7} M and D2-like receptor activation using ropinirole in 10^{-6} M - 10^{-8} M after 14 hours. For motility assessment, a wound healing assay (scrape assay) was used. Cells migrated into the scrape were counted 10, 12, 14 and 16 hours after stimulation. No significant changes between respective receptor activation and the unstimulated control could be observed. The corresponding graph was shown before (Fig. 4.8f).

Treatment	**Migrated cells** (mean)	**SD**	**n**	**p-value**	**significance**
Control	19.12	±4.220	7		
Fenoldopam 10^{-6} M	17.67	±2.723	7	>0.9999	no
Fenoldopam 10^{-7} M	17.87	±4.726	7	>0.9999	no
Ropinirole 10^{-6} M	16.98	±4.018	7	0.2275	no
Ropinirole 10^{-7} M	17.95	± 4.196	7	>0.9999	no
Ropinirole 10^{-8} M	17.79	±3.773	7	>0.9999	no

Table 4.6
Cell motility of RASF under D1-like receptor activation using fenoldopam in 10^{-6} M and 10^{-7} M and D2-like receptor activation using ropinirole in 10^{-6} M - 10^{-8} M after 16 hours. For motility assessment, a wound healing assay (scrape assay) was used. Cells migrated into the scrape were counted 10, 12, 14 and 16 hours after stimulation. No significant changes between respective receptor activation and the unstimulated control could be observed. The corresponding graph was shown before (Fig. 4.8h).

Treatment	**Migrated cells** (mean)	**SD**	**n**	**p-value**	**significance**
Control	21.39	±5.325	7		
Fenoldopam 10^{-6} M	20.07	±3.214	7	>0.9999	no
Fenoldopam 10^{-7} M	20.27	±5.045	7	0.5804	no
Ropinirole 10^{-6} M	19.55	±4.601	7	0.0921	no
Ropinirole 10^{-7} M	20.75	±4.753	7	>0.9999	no
Ropinirole 10^{-8} M	20.22	±4.131	7	>0.9999	no

Table 4.7
Cell motility of OASF under D1-like receptor activation using fenoldopam in 10^{-6} M and 10^{-7} M and D2-like receptor activation using ropinirole in 10^{-6} M - 10^{-8} M after 10 hours. For motility assessment, a wound healing assay (scrape assay) was used. Cells migrated into the scrape were counted 10, 12, 14 and 16 hours after stimulation. No significant changes between respective receptor activation and the unstimulated control could be observed. The corresponding graph was shown before (Fig. 4.8a).

Treatment	**Migrated cells** (mean)	**SD**	**n**	**p-value**	**significance**
Control	12.07	±4.251	8		
Fenoldopam 10^{-6} M	10.88	±2.949	8	>0.9999	no
Fenoldopam 10^{-7} M	11.00	±3.193	8	>0.9999	no
Ropinirole 10^{-6} M	10.69	±2.345	8	0.8029	no
Ropinirole 10^{-7} M	11.51	± 2.562	8	>0.9999	no
Ropinirole 10^{-8} M	11.45	± 1.670	8	>0.9999	no

Table 4.8
Cell motility of OASF under D1-like receptor activation using fenoldopam in 10^{-6} M and 10^{-7} M and D2-like receptor activation using ropinirole in 10^{-6} M - 10^{-8} M after 12 hours. For motility assessment, a wound healing assay (scrape assay) was used. Cells migrated into the scrape were counted 10, 12, 14 and 16 hours after stimulation. No significant changes between respective receptor activation and the unstimulated control could be observed. The corresponding graph was shown before (Fig. 4.8c).

Treatment	**Migrated cells** (mean)	**SD**	**n**	**p-value**	**significance**
Control	14.80	±5.502	7		
Fenoldopam 10^{-6} M	14.18	±3.477	7	>0.9999	no
Fenoldopam 10^{-7} M	14.15	±4.018	7	>0.9999	no
Ropinirole 10^{-6} M	13.53	±2.211	7	0.9927	no
Ropinirole 10^{-7} M	13.62	±2.401	7	>0.9999	no
Ropinirole 10^{-8} M	14.08	±1.931	7	>0.9999	no

Table 4.9
Cell motility of OASF under D1-like receptor activation using fenoldopam in 10^{-6} M and 10^{-7} M and D2-like receptor activation using ropinirole in 10^{-6} M - 10^{-8} M after 14 hours. For motility assessment, a wound healing assay (scrape assay) was used. Cells migrated into the scrape were counted 10, 12, 14 and 16 hours after stimulation. No significant changes between respective receptor activation and the unstimulated control could be observed. The corresponding graph was shown before (Fig. 4.8e).

Treatment	**Migrated cells** (mean)	**SD**	**n**	**p-value**	**significance**
Control	17.85	±6.531	6		
Fenoldopam 10^{-6} M	17.13	±4.109	6	>0.9999	no
Fenoldopam 10^{-7} M	16.52	±4.967	6	>0.9999	no
Ropinirole 10^{-6} M	15.86	±2.945	6	0.448	no
Ropinirole 10^{-7} M	16.05	± 2.026	6	>0.9999	no
Ropinirole 10^{-8} M	16.96	±2.302	6	>0.9999	no

Table 4.10
Cell motility of OASF under D1-like receptor activation using fenoldopam in 10^{-6} M and 10^{-7} M and D2-like receptor activation using ropinirole in 10^{-6} M - 10^{-8} M after 16 hours. For motility assessment, a wound healing assay (scrape assay) was used. Cells migrated into the scrape were counted 10, 12, 14 and 16 hours after stimulation. No significant changes between respective receptor activation and the unstimulated control could be observed. The corresponding graph was shown before (Fig. 4.8g).

Treatment	**Migrated cells** (mean)	**SD**	**n**	**p-value**	**significance**
Control	20.02	±6.025	8		
Fenoldopam 10^{-6} M	19.69	±4.320	8	>0.9999	no
Fenoldopam 10^{-7} M	19.39	± 5.061	8	>0.9999	no
Ropinirole 10^{-6} M	19.38	±3.342	8	>0.9999	no
Ropinirole 10^{-7} M	19.10	±1.715	8	>0.9999	no
Ropinirole 10^{-8} M	19.43	± 1.936	8	>0.9999	no

In the Boyden migration assay, we could observe stronger effects on migration in higher concentrations of DR-agonists. This effect could not be observed in the conducted cell motility assay. The age-dependent effects on cell motility under D1-like and D2-like receptor activation were very similar under all tested concentrations of DR-agonists.

Nevertheless, motility of both OASF and RASF under D1-like and D2-like receptor activation significantly correlates with the age of the patients at surgery (Fig. 4.9 - 4.16). In "younger" patients, both DR agonists led to an increase of cell migration up to 40% in OA and 20% in RA patients under D1-like receptor activation and 100% and 30%, respectively, under D2-like DR activation. In older patients, though, the same receptor activation led to a significant reduction of migration up to 20% under D1-like receptor activation for both OASF and RASF and under D2-like receptor activation up to 50% in OASF and 30% in RASF. Thus, under D2-like receptor activation, both inhibition and induction of cell motility induced by D2-like DR activation was much stronger in SF from OA patients than from RA patients.

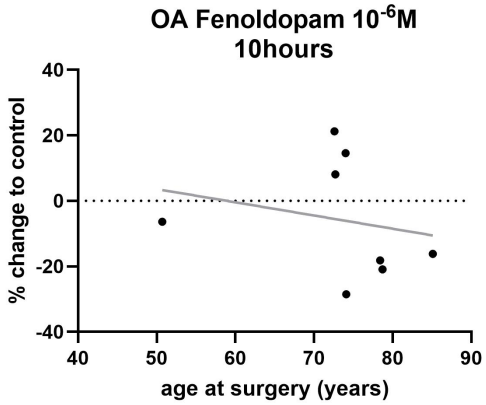
In detail, a significant age-dependent cell motility was observed after 10 hours in RASF under D1-like receptor activation with fenoldopam 10^{-6}M (n=7; r=-0.8214; p=0.034, Fig. 4.9b) and D2-like receptor activation with ropinirole 10^{-6}M (n=7; r= -1.000; p=0.0004, Fig. 4.10b). No significant age-dependent effects were observed in RASF after 10 hours under D1-like receptor activation with fenoldopam 10^{-7}M (n=7; r=-0.6071; p=0.1667, Fig. 4.9d), D2-like receptor activation with ropinirole 10^{-7}M (n=7; r=-0.1786; p=0.7131, Fig. 4.10d) and 10^{-8}M (n=7; r=-0.7500; p=0.0663, Fig. 4.10f).
An age-dependent effect was also observed in OASF after 10 hours under D2-like receptor activation with ropinirole 10^{-7}M (n=8; r=-0.7857; p=0.0279, Fig. 4.10c) and with ropinirole 10^{-8}M (n=8; r=-0.8095; p=0.0218, Fig.4.10e).
There was no significant age-dependent effect on cell motility after 10 hours in OASF under D1-like receptor activation with fenoldopam 10^{-6}M (n=8; r=-0.6190; p=0.1150, Fig. 4.9a), fenoldopam 10^{-7}M (n=8; r=-0.7143; p=0.0576, Fig. 4.9c) and D2-like receptor activation with ropinirole 10^{-6}M (n=8; r=-0.7143; p=0.0576, Fig. 4.10a).

Significant age-dependent cell motility was observed after 12 hours in OASF and RASF under D1-like receptor activation with fenoldopam 10^{-6}M (OASF: n=7; p=0.0123; r=-0.8929, Fig.4.11a and RASF: n=7; p=0.0238; r=-0.8571, Fig.4.11b) and also fenoldopam 10^{-7}M (OASF: n=7; p=0.0238; r=-0.8571,

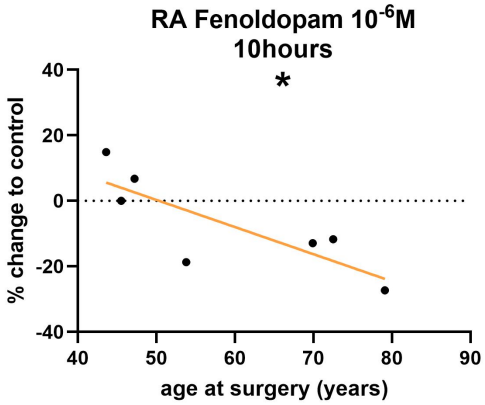
Fig,4.11c and RASF: n=7; p=0.0123; r=-0.8929, Fig.4.11d).
A highly significant age-dependent cell motility was also found in RASF under D2-like receptor activation with ropinirole 10^{-6}M (n=7; p=0.0067; r=-0.9286, Fig. 4.12b) and 10^{-8}M (n=7; p=0.0123; r=-0.8929, Fig. **??**) after 12 hours. Cell motility of OASF was significant under ropinirole 10^{-7}M (n=7; p=0.0238; r=-0.8571, Fig.4.12c) and also with ropinirole 10^{-8}M (n=7; p=0.0341; r=-0.8214, Fig,4.12e). In RASF under D2-receptor activation with ropinirole in 10^{-7}M cell motility was also age-dependent, but not to a significant extent (n=7; p=0.4976; r=-0.3214, Fig. 4.12d). Same was true for OASF motility after treatment with ropinirole 10^{-6}M (Figure a, n=7; p=0.0881; r=-0.7143, Fig.4.12a).

After 14 hours, cell motility was significantly age-dependent in both RASF and OASF under D1-like receptor activation with fenoldopam in 10^{-7}M (RASF: n=7; r=-0.8571; p=0.0238, Fig.4.13d and OASF: n=6; r=-0.8857; p=0.0333, Fig.4.13c). Significant age-dependent changes in cell motility was also observed under D2-like receptor activation with ropinirole in RASF under 10^{-6}M (n=7; r=-0.8571; p=0.0238, Fig.4.14a) and under 10^{-8}M (n=7; r=-0.8571; p=0.0238, Fig4.14e).
There was no significant age-dependent effect on cell motility after 14 hours in OASF and RASF under D1-like receptor activation with fenoldopam in 10^{-6}M (OASF: n=6; r=-0.8286; p=0.0583, Fig.4.13a and RASF: n=7; r=-0.6786; p=0.1095, Fig.4.13b), RASF under D2-like receptor activation with 10^{-7}M (n=7; r=-0.3214; p=0,4976, Fig.4.14d) and OASF under 10^{-6}M (n=6; r=-0.6571; p=0.1750, Fig. 4.14a), 10^{-7}M (n=6; r=-0.7714; p=0.1028, Fig.4.14c) and 10^{-8}M (n=6; r=-0.8286; p=0.0583, Fig.4.14e).

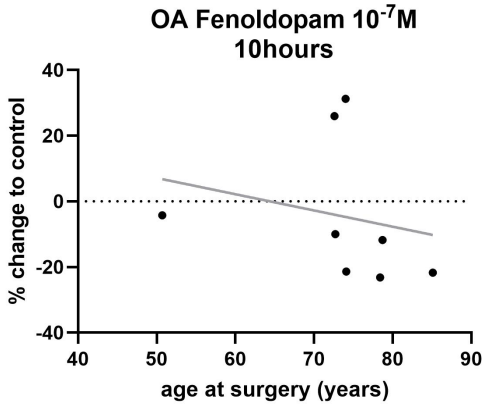
Cell motility of RASF under D1-like receptor activation with fenoldopam in 10^{-7}M (n=7; r=-0.8571; p=0.0238, Fig.4.15d) and D2-like receptor activation with ropinirole in 10^{-6}M (n=7; r=-0.8929; p=0.0123, Fig.4.16b) and 10^{-8}M (n=7; r=-0.8929; p=0.0123, Fig.4.16f) was significantly age-dependent after 16 hours. No significant age-dependent cell motility was observed after 16 hours under D1-like receptor activation with fenoldopam in 10^{-6}M in both OASF (n=8; r=-0.1667; p=0.7033, Fig.4.15a) and RASF (n=7; r=-0.6071; p=0.1667, Fig4.15b) and OASF in 10^{-7}M (n=8; r=-0.6667; p=0.0831, Fig4.15c). No significant age-dependent effects were observed under D2-like receptor activation in OASF in 10^{-6}M (n=8; r=-0.5000; p=0.2162, Fig.4.16a) and RASF in 10^{-7}M (n=7; r=-0.3214; p=0.4976, Fig.4.16d).



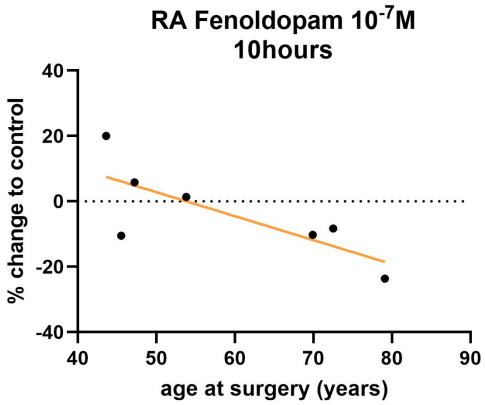
(a) Age-dependent cell motility of OASF under D1-like DR activation with fenoldopam 10^{-6}M after 10 hours (n=8; r=-0.6190; p=0.1150).



(b) Age-dependent cell motility of RASF under D1-like DR activation with fenoldopam 10^{-6}M after 10 hours (n=7; r=-0.8214; p=0.0341).

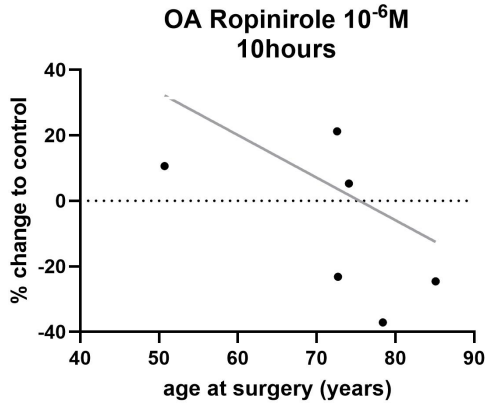


(c) Age-dependent cell motility of OASF under D1-like DR activation with fenoldopam 10^{-7}M after 10 hours (n=8; r=-0.7143; p=0.0576).

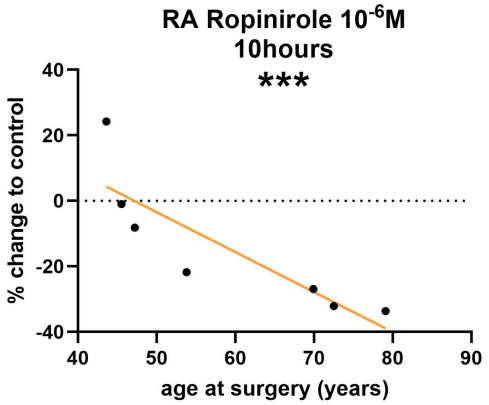


(d) Age-dependent cell motility of RASF under D1-like DR activation with fenoldopam 10^{-7}M after 10 hours (n=7; r=-0.6071; p=0.1667).

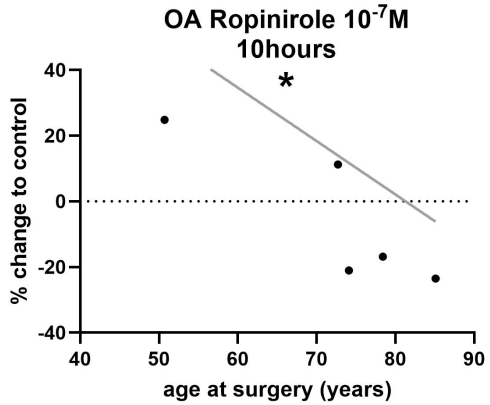
Figure 4.9
Age-dependent cell motility of OASF and RASF under D1-like receptor activation with fenoldopam 10^{-6}M and 10^{-7}M after 10 hours. For cell motility assessment, a scrape assay was performed. Cells that had moved into the gap were counted after 10, 12, 14 and 16 hours and compared to an unstimulated control. The difference is expressed in percent to the control and correlated with the patients' age at surgery, p-values <0.05 were considered as significant.



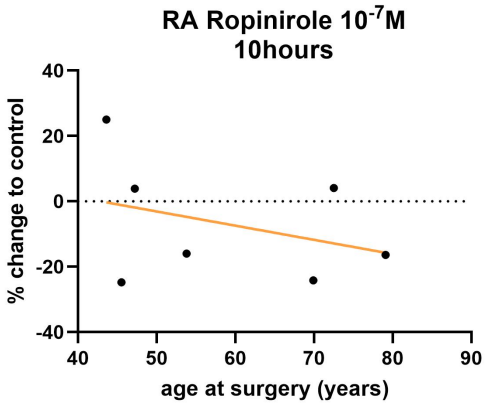
(a) Age-dependent cell motility of OASF under D2-like DR activation with ropinirole 10^{-6}M after 10 hours (n=8; r=-0.7143; p=0.0576).



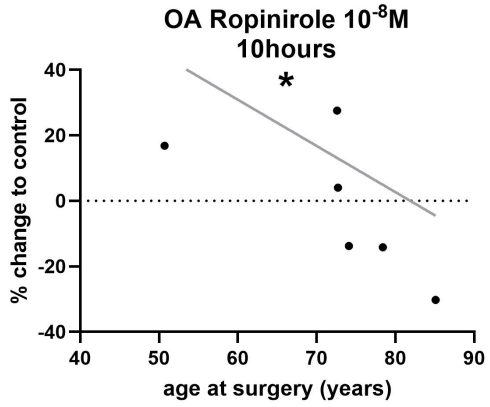
(b) Age-dependent cell motility of RASF under D2-like DR activation with ropinirole 10^{-6}M after 10 hours (n=7; r= -1.000; p=0.0004).



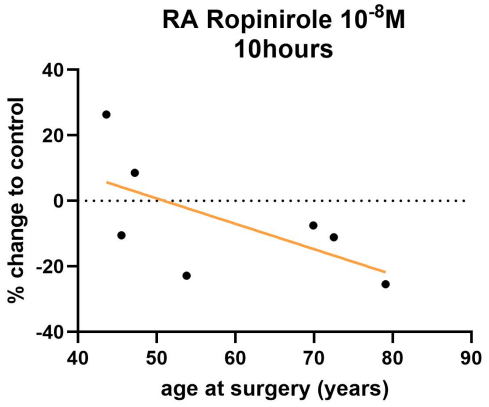
(c) Age-dependent cell motility of OASF under D2-like DR activation with ropinirole 10^{-7}M after 10 hours (n=8; r=-0.7857; p=0.0279).



(d) Age-dependent cell motility of RASF under D2-like DR activation with ropinirole 10^{-7}M after 10 hours (n=7; r=-0.1786; p=0.7131).

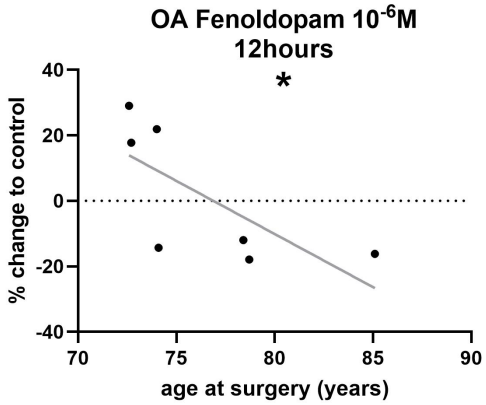


(e) Age-dependent cell motility of OASF under D2-like DR activation with ropinirole 10^{-8}M after 10 hours (n=8; r=-0.8095; p=0.0218).

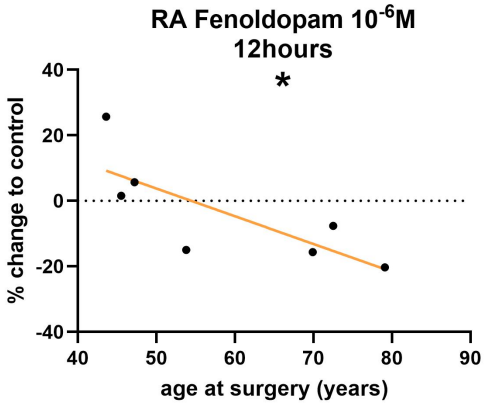


(f) Age-dependent cell motility of RASF under D2-like DR activation with ropinirole 10^{-8}M after 10 hours (n=7; r=-0.7500; p=0.0663).

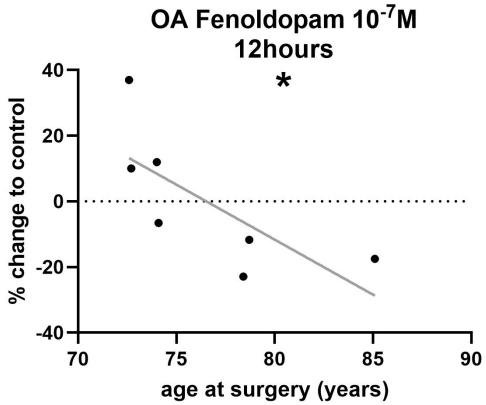
Figure 4.10
Age-dependent cell motility of OASF and RASF under D2-like receptor activation with ropinirole in 10^{-6}M, 10^{-7}M and 10^{-8}M after 10 hours. For cell motility assessment, a scrape assay was performed. Cells that had moved into the gap were counted after 10, 12, 14 and 16 hours and compared to an unstimulated control. The difference is expressed in percent to the unstimulated control and correlated with the patients' age at surgery, p-values <0.05 were considered as significant.



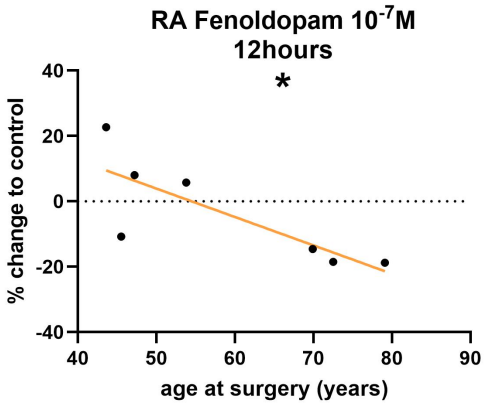
(a) Age-dependent cell motility of OASF under D1-like DR activation with fenoldopam 10^{-6}M after 12 hours (n=7; p=0.0123; r=-0.8929).



(b) Age-dependent cell motility of RASF under D1-like DR activation with fenoldopam 10^{-6}M after 12 hours (n=7; p=0.0238; r=-0.8571).

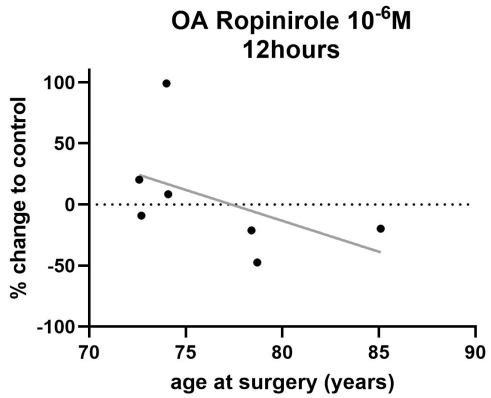


(c) Age-dependent cell motility of OASF under D1-like DR activation with fenoldopam 10^{-7}M after 12 hours (n=7; p=0.0238; r=-0.8571).

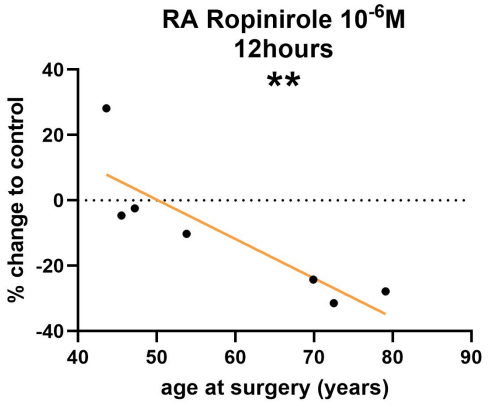


(d) Age-dependent cell motility of RASF under D1-like DR activation with fenoldopam 10^{-7}M after 12 hours (n=7; p=0.0123; r=-0.8929).

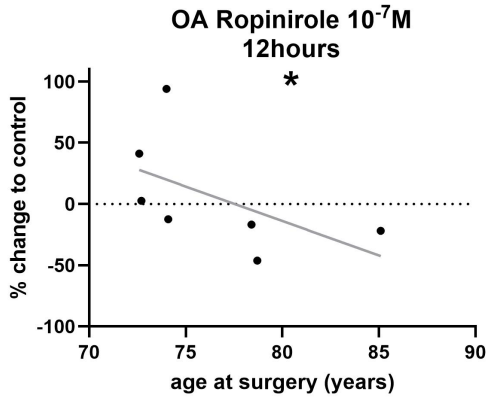
Figure 4.11
Age-dependent cell motility of OASF and RASF under D1-like receptor activation with fenoldopam 10^{-6}M and 10^{-7}M after 12 hours. For cell motility assessment, a scrape assay was performed. Cells that had moved into the gap were counted after 12 hours and compared to an unstimulated control. The difference is expressed in percent to the control and correlated with the patients' age at surgery, P-values <0.05 were considered as significant.



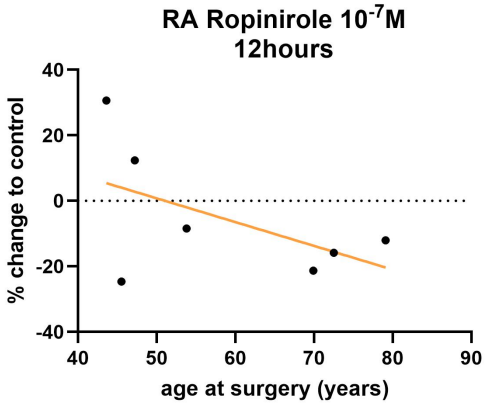
(a) Age-dependent cell motility of OASF under D2-like DR activation with ropinirole 10^{-6}M after 12 hours (n=7; p=0.0881; r=-0.7143).



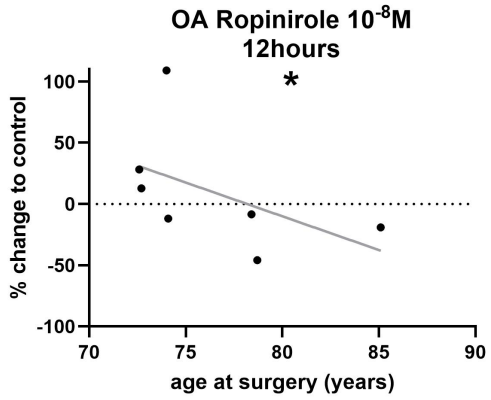
(b) Age-dependent cell motility of RASF under D2-like DR activation with ropinirole 10^{-6}M after 12 hours (n=7; p=0.0067; r=-0.9286).



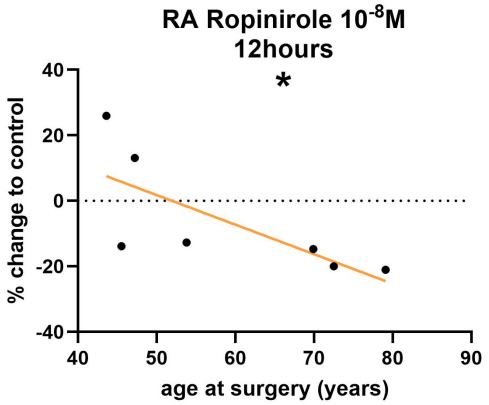
(c) Age-dependent cell motility of OASF under D2-like DR activation with ropinirole 10^{-7}M after 12 hours (n=7; p=0.0238; r=-0.8571).



(d) Age-dependent cell motility of RASF under D2-like DR activation with ropinirole 10^{-7}M after 12 hours (n=7; p=0.4976; r=-0.3214).

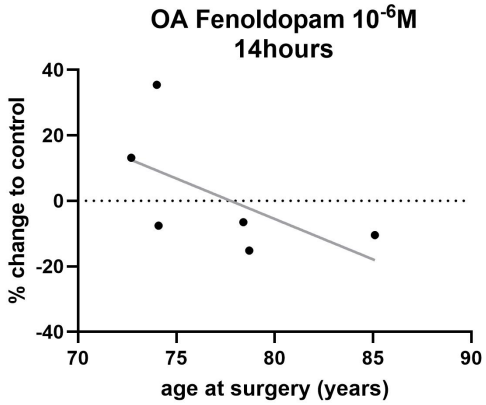


(e) Age-dependent cell motility of OASF under D2-like DR activation with ropinirole 10^{-8}M after 12 hours (n=7; p=0.0341; r=-0.8214).

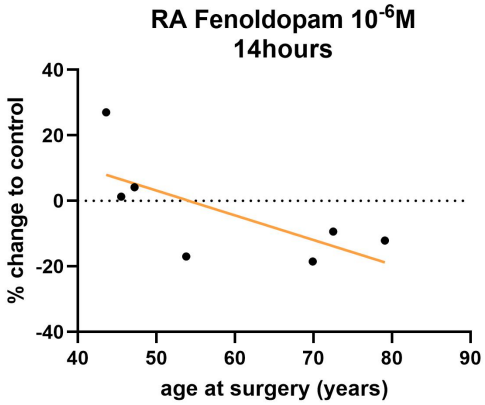


(f) Age-dependent cell motility of RASF under D2-like DR activation with ropinirole 10^{-8}M after 12 hours (n=7; p=0.0123; r=-0.8929).

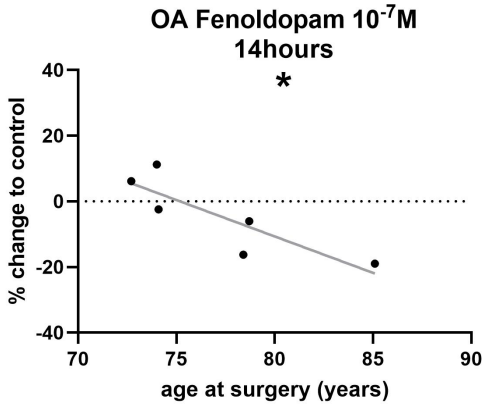
Figure 4.12
Age-dependent cell motility of OASF and RASF under D2-like receptor activation with ropinirole 10^{-6}M, 10^{-7}M and 10^{-8}M after 12 hours. For cell motility assessment, a scrape assay was performed. Cells that had moved into the gap were counted after 10, 12, 14 and 16 hours and compared to an unstimulated control. The difference is expressed in percent to the control and correlated with the patients' age at surgery, p-values <0.05 were considered as significant.



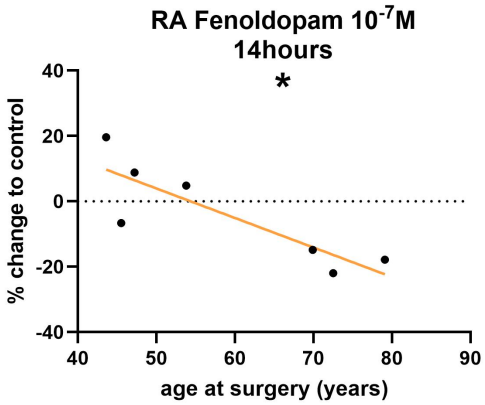
(a) Age-dependent cell motility of OASF under D1-like DR activation with fenoldopam 10^{-6}M after 14 hours (n=6; r=-0.8286; p=0.0583).



(b) Age-dependent cell motility of RASF under D1-like DR activation with fenoldopam 10^{-6}M after 14 hours (n=7; r=-0.6786; p=0.1095).

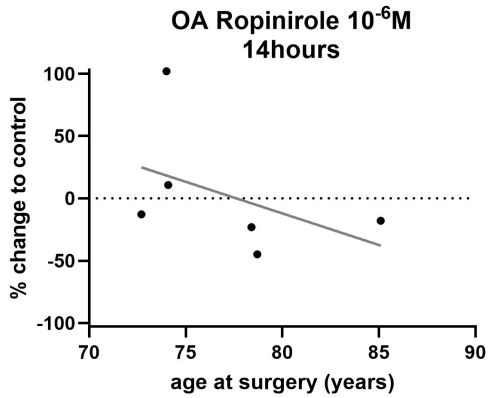


(c) Age-dependent cell motility of OASF under D1-like DR activation with fenoldopam 10^{-7}M after 14 hours (n=6; r=-0.8857; p=0.0333).

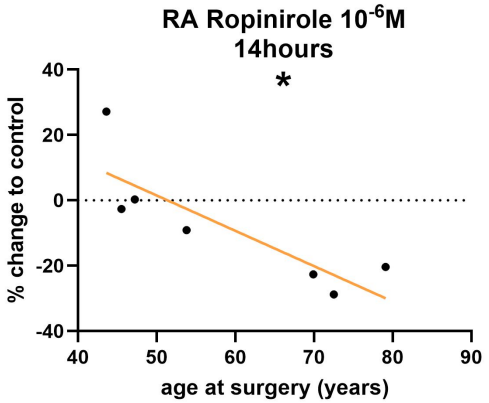


(d) Age-dependent cell motility of RASF under D1-like DR activation with fenoldopam 10^{-7}M after 14 hours (n=7; r=-0.8571; p=0.0238).

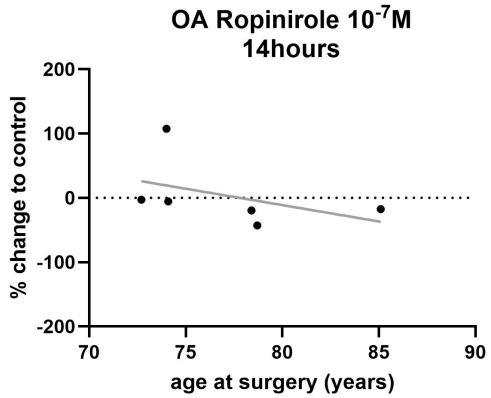
Figure 4.13
Age-dependent cell motility of OASF and RASF under D1-like receptor activation with fenoldopam in 10^{-6}M and 10^{-7}M after 14 hours. For cell motility assessment, a scrape assay was performed. Cells that had moved into the gap were counted after 10, 12, 14 and 16 hours and compared to an unstimulated control. The difference is expressed in percent to the unstimulated control and correlated with the patients' age at surgery, p-values <0.05 were considered as significant.



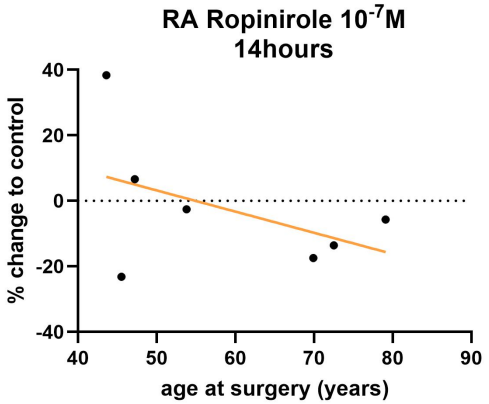
(a) Age-dependent cell motility of OASF under D2-like DR activation with ropinirole 10^{-6}M after 14 hours (n=6; r=-0.6571; p=0.1750).



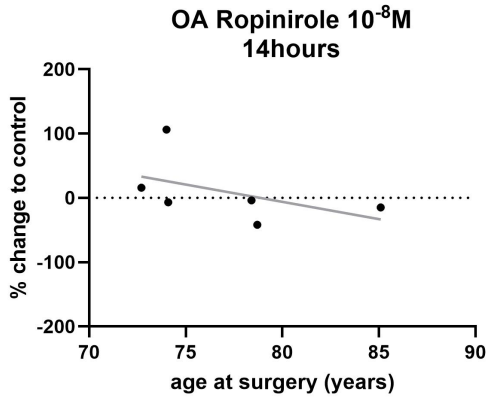
(b) Age-dependent cell motility of RASF under D2-like DR activation with ropinirole 10^{-6}M after 14 hours (n=7; r=-0.8571; p=0.0238).



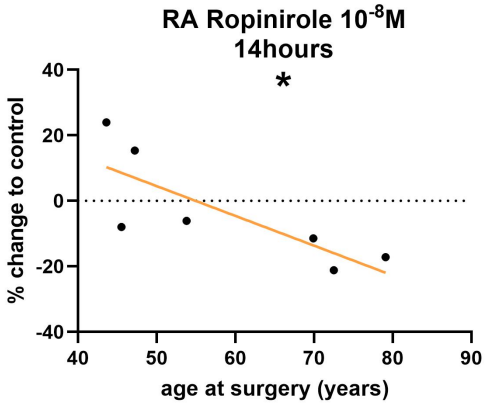
(c) Age-dependent cell motility of OASF under D2-like DR activation with ropinirole 10^{-7}M after 14 hours (n=6; r=-0.7714; p=0.1028).



(d) Age-dependent cell motility of RASF under D2-like DR activation with ropinirole 10^{-7}M after 14 hours (n=7; r=-0.3214; p=0,4976).

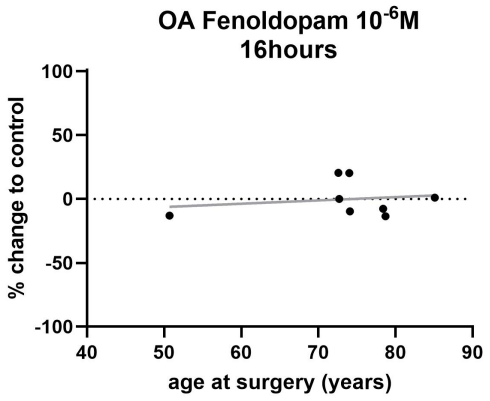


(e) Age-dependent cell motility of OASF under D2-like DR activation with ropinirole 10^{-8}M after 14 hours (n=6; r=-0.8286; p=0.0583).

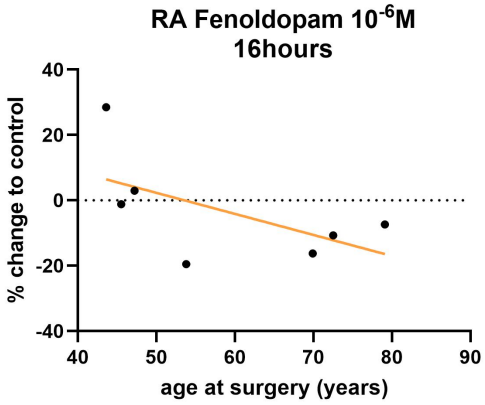


(f) Age-dependent cell motility of RASF under D2-like DR activation with ropinirole 10^{-8}M after 14 hours (n=7; r=-0.8571; p=0.0238).

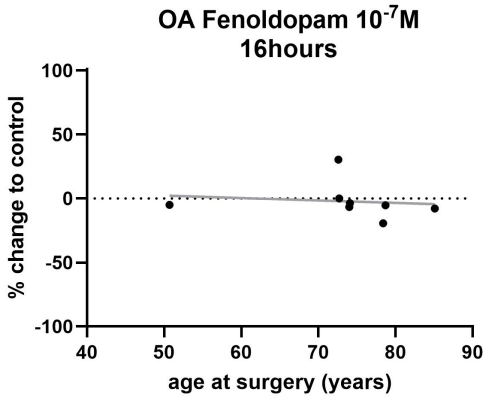
Figure 4.14
Age-dependent cell motility of OASF and RASF under D2-like receptor activation with ropinirole in 10^{-6}M, 10^{-7}M and 10^{-8}M after 14 hours. For cell motility assessment, a scrape assay was performed. Cells that had moved into the gap were counted after 10, 12, 14 and 16 hours and compared to an unstimulated control. The difference is expressed in percent to the unstimulated control and correlated with the patients' age at surgery, p-values <0.05 were considered as significant.



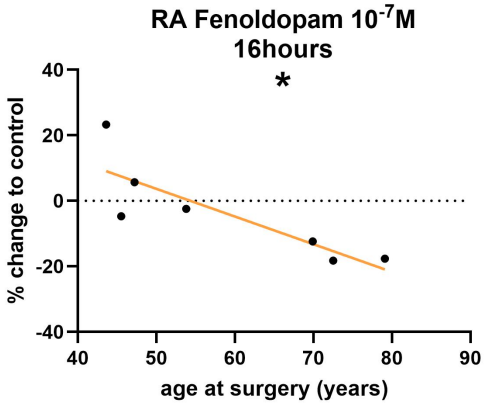
(a) Age-dependent cell motility of OASF under D1-like DR activation with fenoldopam 10^{-6}M after 16 hours (n=8; r=-0.1667; p=0.7033).



(b) Age-dependent cell motility of RASF under D1-like DR activation with fenoldopam 10^{-6}M after 16 hours (n=7; r=-0.6071; p=0.1667).

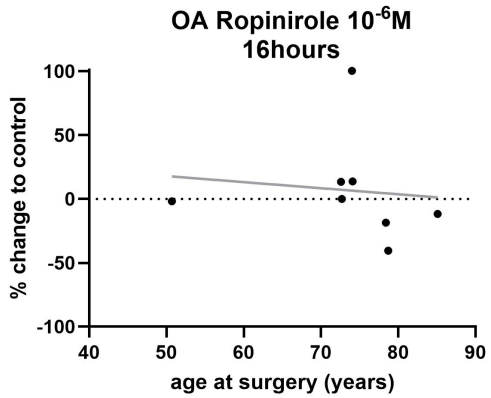


(c) Age-dependent cell motility of OASF under D1-like DR activation with fenoldopam 10^{-7}M after 16 hours (n=8; r=-0.6667; p=0.0831).

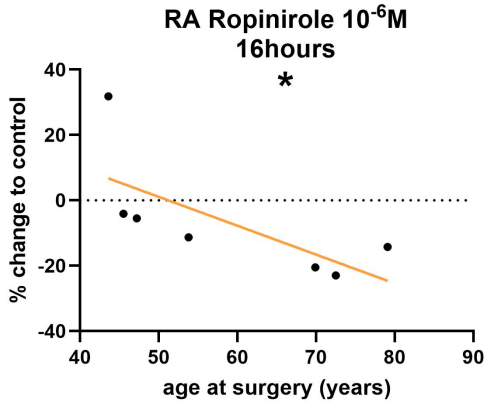


(d) Age-dependent cell motility of RASF under D1-like DR activation with fenoldopam 10^{-7}M after 16 hours (n=7; r=-0.8571; p=0.0238).

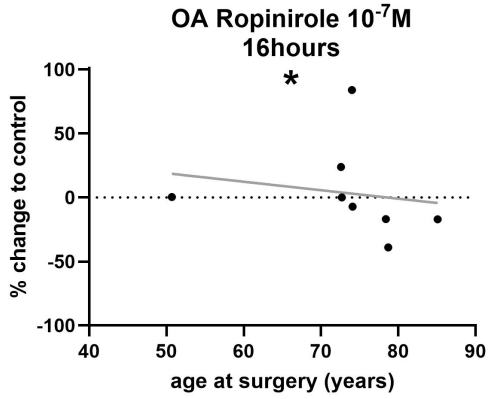
Figure 4.15
Age-dependent cell motility of OASF and RASF under D1-like receptor activation with fenoldopam in 10^{-6}M and 10^{-7}M after 16 hours. For cell motility assessment, a scrape assay was performed. Cells that had moved into the gap were counted after 10, 12, 14 and 16 hours and compared to an unstimulated control. The difference is expressed in percent to the unstimulated control and correlated with the patients' age at surgery, p-values <0.05 were considered as significant.



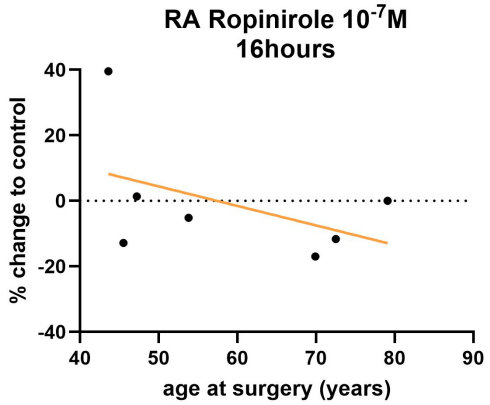
(a) Age-dependent cell motility of OASF under D2-like DR activation with ropinirole 10^{-6}M after 16 hours (n=8; r=-0.5000; p=0.2162).



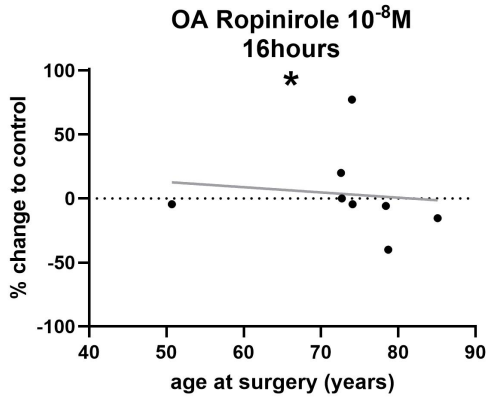
(b) Age-dependent cell motility of RASF under D2-like DR activation with ropinirole 10^{-6}M after 16 hours (n=7; r=-0.8929; p=0.0123).



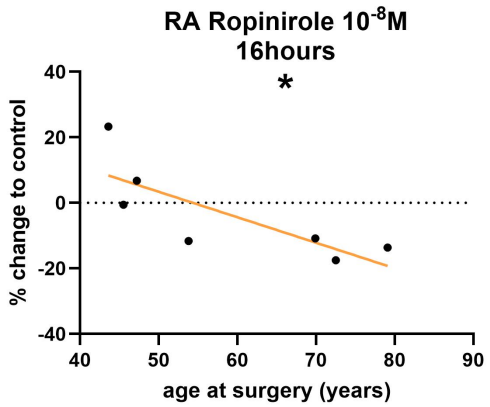
(c) Age-dependent cell motility of OASF under D2-like DR activation with ropinirole 10^{-7}M after 16 hours (n=8; r=-0.8095; p=0.0218).



(d) Age-dependent cell motility of RASF under D2-like DR activation with ropinirole 10^{-7}M after 16 hours (n=7; r=-0.3214; p=0.4976).



(e) Age-dependent cell motility of OASF under D2-like DR activation with ropinirole 10^{-8}M after 16 hours (n=8; r=-0.7619; p=0.0368).



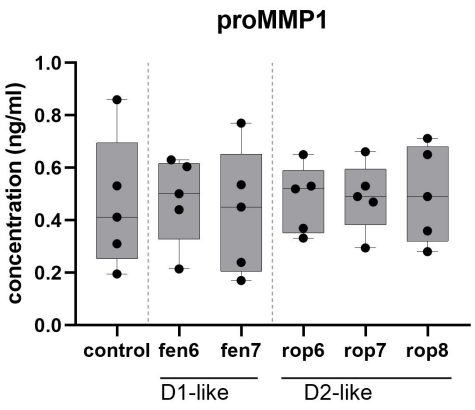
(f) Age-dependent cell motility of RASF under D2-like DR activation with ropinirole 10^{-8}M after 16 hours (n=7; r=-0.8929; p=0.0123).

Figure 4.16
Age-dependent cell motility of OASF and RASF under D2-like receptor activation with ropinirole in 10^{-6}M, 10^{-7}M and 10^{-8}M after 16 hours. For cell motility assessment, a scrape assay was performed. Cells that had moved into the gap were counted after 10, 12, 14 and 16 hours and compared to an unstimulated control. The difference is expressed in percent to the unstimulated control and correlated with the patients' age at surgery, p-values <0.05 were considered as significant.

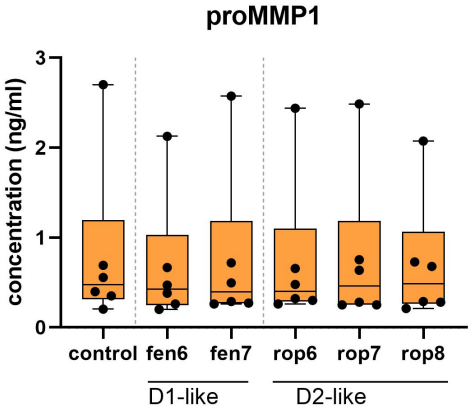
4.4 D1-like and D2-like receptor activation has no significant effect on IL6, proMMP1 and MMP3 release in both RASF and OASF

The localisation of DR nearby the invasion zone and the lining layer, together with the previously shown results from migration and motility assays, suggest a pivotal role of DR also in matrix degradation and cytokine production.
Therefore, we performed ELISA (Enzyme-linked Immunosorbent Assay) for MMP3 and proMMP1, well-known and prominent tissue-destructing enzymes, using supernatants from RASF and OASF previously stimulated with the already previously used different concentrations of fenoldopam and ropinirole. As dopamine itself was shown to significantly reduce IL6-release from RASF,[207] we evaluated whether this effect is rather dependent on D1-like or D2-like receptor activation.

Concerning both proMMP1 and MMP3, no significant changes under D1-like and D2-like receptor activation could be observed in both RA (n=6) and OA (n=4) patients (for proMMP1 in Fig. 4.17, Fig.4.18, Tbl.4.12, Tbl. 4.11, for MMP3: Fig. 4.18, Tbl. 4.13 and Tbl. 4.14). Plus, no differences between D1-like and D2-like receptor activation could be observed. Similar results were obtained when IL6 concentration in RASF and OASF supernatants were assessed after D1-like and D2-like receptor activation. Even though D2-like receptor activation with ropinirole 10^{-6}M led to a significant increase in IL6 release, this effect is most likely not of any clinical relevance and rather due to the limited number of samples available for this experiment (Figure 4.19, Tbl. 4.15 and Tbl. 4.16).
Also, concentrations of proMMP1 and MMP3 did not significantly differ between RA and OA patients. In RA, one patient showed much higher concentrations of proMMP1 compared to other RA patients. The available information about this patient did not show any reasons (e.g., active inflammatory flare) to explain this difference.
Regarding IL6, RA patients showed higher concentrations IL6 compared to OA patients, but also not to a significant extent. Different concentrations of proMMP1, MMP3 and IL6 were also analyzed for age-dependency, but no significant differences could be observed (data not shown). This was also in consequence of the small number of patients (n = 4-6).

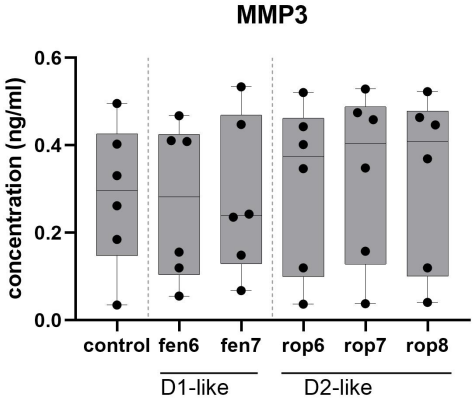


(a) proMMP-1 release of OASF under D1-like and D2-like receptor activation.

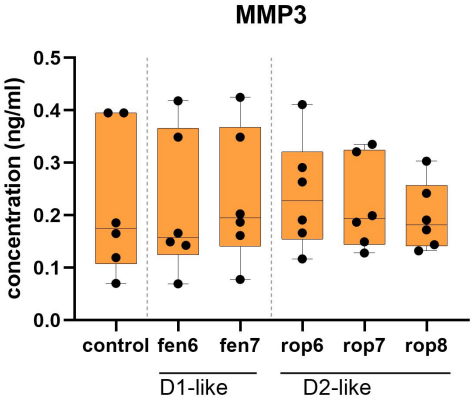


(b) proMMP-1 release of RASF under D1-like and D2-like receptor activation.

Figure 4.17
ProMMP1 release of OASF (n=4) and RASF (n=6) under D1-like and D2-like receptor activation in different concentrations. No significant changes between respective receptor activation and proMMP1 release could be observed in OASF or RASF compared to an unstimulated control. D1-like receptors were activated by fenoldopam (fen) in concentrations of 10^{-6}M (fen6) and 10^{-7}M (fen7). For D2-like receptor activation, ropinirole in 10^{-6}M (rop6), 10^{-7}M (rop7) and 10^{-8}M (rop8) was used. For quantification, ELISAs were used.

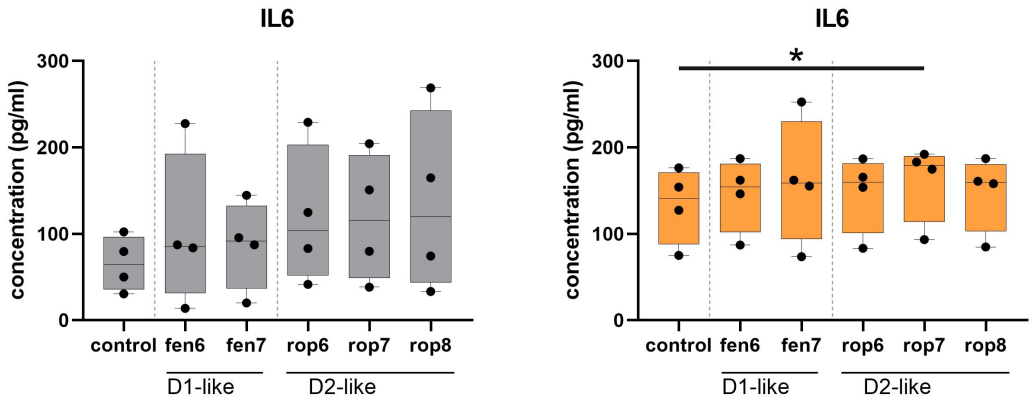


(a) MMP-3 release of OASF under D1-like and D2-like receptor activation.



(b) MMP-3 release of RASF under D1-like and D2-like receptor activation.

Figure 4.18
MMP3 release of OASF (n=6) and RASF (n=6) under D1-like and D2-like receptor activation in different concentrations measured by ELISA. No significant changes between the respective receptor activation and change in MMP3 release could be observed in both OASF or RASF. D1-like receptors were activated by fenoldopam (fen) in concentrations of 10^{-6}M (fen6) and 10^{-7}M (fen7). For D2-like receptor activation, ropinirole in 10^{-6}M (rop6), 10^{-7}M (rop7) and 10^{-8}M (rop8) was used.



(a) IL-6 release of OASF under D1-like and D2-like receptor activation.

(b) IL-6 release of RASF under D1-like and D2-like receptor activation.

Figure 4.19
IL6 release of OASF (n=4) and RASF (n=4) under D1-like and D2-like receptor activation in different concentrations measured by ELISA. Only in D2-like receptor activation with ropinirole 10^{-6}M a significant increase in IL6-release could be observed. Other concentrations tested did not show significant changes between respective receptor activation and change in IL6-release in OASF or RASF. D1-like receptors were activated by fenoldopam (fen) in concentrations of 10^{-6}M (fen6) and 10^{-7}M (fen7). For D2-like receptor activation, ropinirole in 10^{-6}M (rop6), 10^{-7}M (rop7) and 10^{-8}M (rop8) was used.

Table 4.11 proMMP1 release of RASF under D1-like and D2-like receptor activation compared to an unstimulated control. For quantification, ELISA was used. P-values <0.05 were considered as significant.

Treatment	Concentration (ng/ml)	SD	n	p-value	significance
Control	0.8166	±0.9385	6		
Fenoldopam 10^{-6} M	0.6840	±0.7255	6	0.3204	no
Fenoldopam 10^{-7} M	0.7683	± 0.9026	6	>0.9999	no
Ropinirole 10^{-6} M	0.7424	±0.8442	6	>0.9999	no
Ropinirole 10^{-7} M	0.7761	±0.8650	6	>0.9999	no
Ropinirole 10^{-8} M	0.7104	±0.7033	6	>0.9999	no

Table 4.12 proMMP1 release of OASF under D1-like and D2-like receptor activation compared to an unstimulated control. For quantification, ELISA was used. P-values <0.05 were considered as significant.

Treatment	Concentration (ng/ml)	SD	n	p-value	significance
Control	0.4617	±0.2548	5		
Fenoldopam 10^{-6} M	0.4780	±0.1661	5	>0.9999	no
Fenoldopam 10^{-7} M	0.4331	±0.2402	5	>0.9999	no
Ropinirole 10^{-6} M	0.4803	±0.1293	5	>0,9999	no
Ropinirole 10^{-7} M	0.4892	±0.1318	5	>0.9999	no
Ropinirole 10^{-8} M	0.4983	± 0.1841	5	0.6410	no

Table 4.13 MMP3 release of RASF under D1-like and D2-like receptor activation compared to an unstimulated control. For quantification, ELISA was used. P-values <0.05 were considered as significant.

Treatment	Concentration (ng/ml)	SD	n	p-value	significance
Control	0.2217	±0.1400	6		
Fenoldopam 10^{-6} M	0.2157	±0.1359	6	>0.9999	no
Fenoldopam 10^{-7} M	0.2338	±0.1286	6	>0.9999	no
Ropinirole 10^{-6} M	0.2400	±0.1053	6	0.6141	no
Ropinirole 10^{-7} M	0.2200	± 0.08759	6	>0.9999	no
Ropinirole 10^{-8} M	0.1974	±0.06465	6	>0.9999	no

Table 4.14 MMP3 release of OASF under D1-like and D2-like receptor activation compared to an unstimulated control. For quantification, ELISA was used. P-values <0.05 were considered as significant.

Treatment	**Concentration** (ng/ml)	**SD**	**n**	**p-value**	**significance**
Control	0.2853	±0.1635	6		
Fenoldopam 10^{-6} M	0.2698	±0.1789	6	>0.9999	no
Fenoldopam 10^{-7} M	0.2796	± 0.1779	6	>0.9999	no
Ropinirole 10^{-6} M	0.3115	±0.1912	6	>0.9999	no
Ropinirole 10^{-7} M	0.3345	± 0.1963	6	>0.9999	no
Ropinirole 10^{-8} M	0.3274	±0.1990	6	>0.9999	no

Table 4.15 IL6 release of RASF under D1-like and D2-like receptor activation compared to an unstimulated control. For quantification, ELISA was used. P-values <0.05 were considered as significant.

Treatment	**Concentration** (pg/ml)	**SD**	**n**	**p-value**	**significance**
Control	133.2	±43.68	4		
Fenoldopam 10^{-6} M	145.7	±42.48	4	0.9294	no
Fenoldopam 10^{-7} M	161.0	±73.20	4	0.6529	no
Ropinirole 10^{-6} M	147.4	±44.86	4	0.6529	no
Ropinirole 10^{-7} M	160.9	±45.56	4	0.0229	**yes**
Ropinirole 10^{-8} M	147.7	±43.89	4	0.2939	no

Table 4.16 IL6 release of OASF under D1-like and D2-like receptor activation compared to an unstimulated control. For quantification, ELISA was used. P-values <0.05 were considered as significant.

Treatment	**Concentration** (pg/ml)	**SD**	**n**	**p-value**	**significance**
Control	65.68	±31.65	4		
Fenoldopam 10^{-6} M	103.1	±89.59	4	>0.9999	no
Fenoldopam 10^{-7} M	86.93	±51.16	4	>0.9999	no
Ropinirole 10^{-6} M	119.7	±80.51	4	0.1167	no
Ropinirole 10^{-7} M	118.4	±73.81	4	0.4449	no
Ropinirole 10^{-8} M	135.4	±104.5	4	0.2939	no

Chapter 5

Discussion

Despite substantial progress in the field of therapeutic options in RA, a significant part of RA patients still do not achieve permanent disease remission and still suffer from progressive joint destruction. In addition, patients with high disease activity are more likely to suffer from severe comorbidities such as diabetes and myocardial infarction with subsequent higher rates of hospitalization and shorter life expectancy.
As dopamine was previously shown to be a crucial mediator in inflammatory - especially autoinflammatory - processes and specifically in RA, we further investigated the effects of dopaminergic signaling on synovial fibroblasts of patients suffering from RA and OA.

For the first time, we could show, that all investigated DR are expressed nearby the invasion zone of the synovium into the adjacent bone and are therefore likely involved in RASF pathophysiology.
Specifically, our findings showed that especially D1DR, D2DR and D5DR were significantly stronger expressed in the invasion zone of RA patients compared to the sublining tissue.[11] D4DR staining of RA synovium and invasion zone was conducted in parallel at the IfaDo laboratory by Silvia Capellino in Dortmund using an established and comparable protocol. Here, D4DR staining intensity was shown to be stronger nearby the invasion zone compared to the sublining layer.[11]
Our results also reflect the previously published results from Capellino et al.,[207] in which all DR subtypes were found to be expressed in RA and OA synovial tissue. Of note, DR were shown to be stronger expressed in RA synovium than in OA..[207]
The invasion zone in the synovium is unique for RA. Synovial fibroblasts aggressively destroy cartilage and in later stages also osseous structures. As both D1-like and D2-like receptors subtypes were found in this tissue compart-

ment, this might explain the observed similar effects of D1-like and D2-like DR activation on cell migration and motility.
Moreover, all investigated DR were stronger expressed in the lining layer than in the sublining tissue. This was both true for RA and OA synovial tissue. However, in RA patients, D1DR, D2DR and D5DR were evenstronger expressed compared to OA, confirming previous results.[207]

As DR density seems to be enhanced in regions of high cellular activity (the lining layer) and migration of SF (invasion zone), we suggested that dopamine and its intracellular pathways crucially contribute to RA pathogenesis.
RA patients show elevated levels of cytokines and dopamine in the syovial fluid and blood,[216] suggesting a potential involvement of dopamine in cytokine release into blood and synovial fluid. Also dopamine potentially acts on synoviocytes in the synovial lining layer in a paracrine manner, perpetuating dopaminergic pathways in RASF and may further promote DR expression on RASF.
Until now, it is still unclear, whether dopamine and high DR expression on RASF has rather pro- or anti-inflammatory effects and whether matrix remodeling caused by RASF is affected by this mechanism. In our results, we could not observe any significant change in MMP3 and proMMP1-release under D1-like and D2-like receptor agonists. Although IL6-release was significantly increased in RASF under D2-like receptor activation, this is most likely due to the limited amount of samples available for this experiment. In contrast to these results, Capellino et al. showed in 2014,[207] that treatment of RASF with dopamine for 48 hours led to a significant decrease of IL6 release in RASF but not OASF, which is opposite to our observed effects. Dopamine and dopamine agonists were also previously shown to inhibit the upregulation of IL6 from macrophages/monocytes in early stages of inflammation.[257] Keren et al. also showed on T-cells, that IL6-release was significantly reduced after incubation with fenoldopam in 10-6M for 48 hours[197]

On the other hand, dopamine promoted IL6 release via D2DR in cells obtained from the glomerulosa cells of the kidney[257] and also macrophages[258] As cytokine release via DR was shown to be mainly mediated on mRNA level,[12] 24 hours of incubation might have been too short in order to achieve significant effects and has to be extended to 48 hours as performed by Keren et al.[197] and Capellino et al.[207] in order to test whether DA signaling is a slow process and longer stimulation is necessary to see stronger alterations in cytokine release. Furthermore, we did not see any dose-dependent effects by using specific D1-like and D2-like receptor agonists in different concentrations, which is also in

contrast to previous results by Silvia Capellino.[207]

However, increased expression of DR in the lining layer was not only observed in RA but also in OA patients, even though to a lower extent in most investigated receptor subtypes.
Osteoarthritis is no primarily inflammatory disease, but exacerbations of arthritis with local inflammation are frequent, which might be due to the upregulated dopamine receptor density in the synovium. As only far progressed stages of disease with necessary knee arthroplasty were included into this study (end-stage disease), OA patients might present higher inflammatory - not autoinflammatory - stages compared to early stages of OA, which were not available for this project.

Furthermore, we could not investigate healthy or rather "non-inflamed" synovial tissue in order to assess "normal" dopamine receptor density on SF. This is also true for the other experiments and certainly restricts the validity of the experiments. As we had to rely on samples from the cell and tissue bank, there were only limited options of generating a representative age distribution for both RA and OA. Owing to better treatment options, RA patients in our experiments were only 10 years younger in average than OA patients, but nevertheless, we were not able to get the same age distribution in RA and OA. Concerning IHC, the OA and RA patients included into the experiment were about the same age, so we could not investigate a possible age-related DR-distribution or expression within the synovium.
As DR expression was highly increased in the RA invasion zone, cell migration and motility were evaluated. Although, no significant effects were observed when comparing RASF and OASF migration and motility under DR activation with the unstimulated control of both RA and OA patients, cell migration and also motility significantly correlated with the age of patients at surgery. Both D1-like and D2-like receptor activation led to a significant increase of cell migration and motility in younger patients (< 75 years), whereas a significant decrease was observed in patients older than 75 years.
This suggests, that the same receptor activation leads to opposite effects on cell migration and motility in younger vs older patients. As this was observed in both RA and OA patients, this seems to be no specific phenomenon in RA but rather an age-dependent effexct of dopamine receptors. Stronger effects were observed in RA, possibly caused by the higher DR density on RASF.

In the existing literature, dopamine and specific dopamine receptor activation

has been shown to both promote and inhibit cell migration. In most experiments immune cells, especially T-cells have been investigated. However, no experiments using RASF have been conducted yet. Surprisingly, we did not observe any differences between D1-like and D2-like receptor activation, although those two receptor subtypes are commonly thought to have opposite effects by acting via an increase or decrease of intracellular cAMP concentrations. To our knowledge, this has also not been described before. In order to quantify age-dependent DR density on RASF and OASF, FACS (fluorescence activated cell sorting) stainings have been performed by Silvia Capellino. Here, it could be shown that density of D1DR, D2DR and D4DR on RASF but not OASF significantly decreases with age.[11]
This has already been shown in other cells in- and outside the CNS[247–249,254] and appears to be also true for RASF. This might explain different - although not opposite - effects of DR activation in older vs. younger patients. Interestingly, even though OA patients reacted the same way as RA patients in our experiments, they were not affected by age-dependent DR-loss. This suggests, that the observed age-dependent changes are not only associated with altered receptor density with increasing age. In order to see whether our results might be due to a lower receptor sensitivity for DR agonists with increasing age, the group of Silvia Capellino also performed a X-CELLigence real-time cell analysis, but no age-dependent change concerning the respective receptor response was observed.[11]

Dopamine receptors with subsequent intracellular pathways are known to be rather complex, and explanations for our results can be found on many levels of dopamine signaling, ranging from receptor density to responsiveness, hetero- and homomers, to differences in intracellular signaling. Several possible explanations, as discussed below, have not been investigated on RASF yet, which is why it is difficult to transfer obtained results from other cell types to SF.

Previous studies showed that prednisolone treatment of RA before joint replacement surgery significantly reduced cytokine release in RASF. Similarly, cell density of TH+ cells in RA synovial tissue was significantly lower.[112] Of note, almost all RA patients we included into this study were treated with prednisolone before surgery.
Unfortunately, we did not have access to medication of all investigated patients included. Prednisolone and modern DMARD treatment might explain, why differences between OASF and RASF were not as significant as expected. Also, the number of patients included to the experiments could be further increased

with equal distribution of age and detailed information about the medical history in order to strengthen the observed results.
However, in our experiments, disease duration did not correlate with the migration of RASF. This might be due to the fact, that there were only few patients, of whom we knew the actual duration of disease, but it also might be due to the highly variable progress of RA.

It was recently described, that D2DR expression on B-cells of RA patients is inversely correlated with disease activity and CRP levels.[259] This suggests the idea that the higher the disease activity, the lower D2DR expression on the cell surface. After treatment with DMARDs, expression of D2DR on B-cells significantly increased in this study. Thus, DR-expression seems to be dependent from cell environment.
Whether this is also true for RASF, still needs to be elucidated, as it might explain the observed opposite effects. As already mentioned above, DR density decreases with increasing age, which might be a reason why more and more patients do not achieve permanent remission with increasing age, as DR density can not be restored.
Recent research showed a different expression of D1DR on B-cells in male and female RA patients. Female RA patients showed a significantly higher expression of D1DR whereas in male RA patients, D1DR, expression on B-cells was reduced compared to healthy controls.[260] Furthermore, D1DR receptor density on B-cells correlates with RA disease duration in female RA patients, but not in male.

These new findings add exciting aspects concerning further research and also future treatment options for RA. With respect to our RA patients included into the experiments, we could not find significant differences between male and female patients, but we only had very few male RA patients. In OA, more male patients were included, but no differences were observed (data not shown).
Wieber et al. also demonstrated, that D1DR expression on B-cells correlates with disease duration in female RA patients but not male RA patients. D1DR -expression on B-cells was further correlated with disease disability.[260]
Although Wieber et al. could not observe any age-dependent DR-expression on B-cells, it illustrates, that DR expression is altered by many factors and DR expression also seems to change during the course of disease.

Dopamine receptors are also known to form hetero- and homomers with other DR subtypes and also other receptor types like adenosine receptors.

This can significantly change the effects of the respective receptor subtype. For example, it was shown that ropinirole has much higher potency on D2DR/D3DR heterodimers than on the single D2DR or D3DR receptor.[165] Dopamine receptor heteromers have mainly been investigated in the CNS, which is why heteromers especially on RASF need to be further investigated in order achieve a better understanding of the results.
It has also been shown by Torvinen et al., that D3DR form heteromers with the adenosine receptor 2A. In this heteromer, the adenosine receptor modulates affinity and signaling of the dopamine receptor.[166] Adenosine receptors have also been found in the RA synovium[261] and also underly an age-dependent change in expression[262] and metabolism.[263]
Combined treatment of D1DR agonists and adenosine A1 agonists has also been shown to cause significantly lower accumulation of cAMP compared to D1DR treatment alone.,[164] which further emphasizes the importance of further research of DR hetero- and homomers.
Adenosine receptors are of special interest in this topic, as MTX, the first-line DMARD, acts among other targets via adenosine receptors. Like this, MTX is likely to influence DR signaling by potentially activating or also inhibiting heteromers between adenosine and dopamine receptors.

With respect to intracellular effects of dopamine receptor activation, GPCR were recently found to be able to switch from Gα_s to Gα_i signaling in RA synovium.[264] This was not specifically shown for DR, but it gives another possible explanation, why we observed similar effects under both D1-like (Gα_s coupled receptor) and D2-like (G$\alpha_i$coupled receptor) receptor agonists.
This might also explain, how DR are able to cause such a variety of effects on different cells and why it is so difficult to capture the whole image of DR signaling. Further experiments especially concerning the intracellular pathways following DR activation are needed in order to support this hypothesis.
At the same time, patients suffering from juvenile arthritis have been shown to suffer from an impaired intracellular cAMP metabolism.[84] As DR are commonly known to act via G$\alpha_{s/i}$ and altered cAMP levels, this could also affect DR expression on the cell surface and disturb intracellular pathways following dopamine receptor activation.
Different intracellular pathways following DR activation have been described in the literature, mainly those operative in the CNS. For example, DR activation has been shown to affect calcium channels independently from GPCR activation. Calcium channels in turn are involved in several intracellular processes finally leading to cell migration.[265,266] Our results suggest, that the intracellular

pathways in RA are different from well-known G-protein coupled Gα pathways and need to be further elucidated in order to improve RA treatment.

Among modified DR-expression and intracellular pathways, another aspect is the age-dependent alteration of the cytoskeleton. When immature human CD34+ cells (haemopoietic progenitor cells) were treated with the specific D1-like receptor agonist 7-OH-DPAT, this led to a significant rearrangement of the cytoskeleton, which is known to be also altered with increasing age[267] and therefore might also - at least partly - explain the observed age-dependent differences in RASF and OASF migration and motility.
Dopamine is also of special interest in other research fields like nephrology. Here, Zbroch at al. found out, that there is a reverse correlation between renalase concentrations (an enzyme that degrades catecholamines) and age in patients needing hemodialysis.[254] This puts focus rather on altered catecholamine degradation with increasing age.

Even though these results are significantly contributing to a better understanding of peripheral DR signaling and metabolism, they only partly explain our results.
Regarding age-dependent opposite effects of dopamine on DR activation, little is known yet. Interesting results regarding this topic were obtained from experiments with rats: In order to investigate dopamine activity especially in aged rats, K+-stimulated release of dopamine in the brain was measured. In older rats (12 month) dopamine release was decreased by 50% compared to younger (3 month) rats. In turn, when treated with ouabin (a Na-K-ATPase inhibitor), dopamine release was significantly decreased in in young rats, but older rats showed a significant increase up to 250 %.[268]
Similarly, it could be shown, that sodium excess leads to increased dopamine levels in urine in adults, whereas opposite effects with reduced dopamine levels were observed in younger people.[269] Additional experiments showed, that treatment with dopamine antagonists leads to natriuresis in young people whereas it leads to sodium retention in older people.[269]
Adverse age-dependent effects of dopamine, as we observed in our conducted experiments, have been shown before and do not seem to be RA-exclusive. But to our current knowledge, definitive explanations for this phenomenon are still missing.

Taken together, our experiments contributed new insights into peripheral dopaminergic effects on RASF especially in RA-associated pathophysiologic as-

pects. We could show, that dopamine receptors are present in the invasion zone of RA synovium and dopamine receptor activation leads to an age-dependent effect on migration of RASF and OASF.
Based on our obtained results we suggest, that RA drug therapy has to be adapted to the patient's age, as the dopaminergic response alters with increasing age. Age-dependent opposite effects of DR activation on cells have been described before, but associated intracellular pathways especially concerning the peripheral dopaminergic system are still not fully understood.

Chapter 6

Summary

Rheumatoid Arthritis is a chronic autoimmune disease, with inflammation of the joints and several extraarticular manifestations. Even though 1% of the world's population is affected and a majority of patients is in working-age, there is still no cure for this disease.
Chronic inflammation of the small joint of hands and feet are typical manifestations, which leads to hyperproliferation of the inflamed synovium. Synovial fibroblasts transfom into aggressive cells, that invade into adjacent bone and cartilage structures, leading to progressive damage of the joint. Furthermore, they secrete immunomodulatory factors and show uninhibited proliferation.

In recent studies, dopamine has been shown to be synthesized in RASF, and all DR subtypes have been found on RASF. Compared to OA, dopamine receptors were overexpressed, pointing towards a significant role of DR in RA.
For the first time we could show, that D1DR, D2DR, D3DR and D5DR are present in the invasion zone. Here, D1DR, D2DR and D5DR were stronger expressed in the invasion zone compared to the sublining layer. This suggests a distinct contribution of the dopaminergic system concerning invasion of RASF into cartilage and bone. All tested DR were also stronger expressed in the lining layer compared to the sublining layer in RA and OA synovium and all tested DR were stronger expressed in RA than in OA synovium. In contrast to previous publications, we could not find relevant alterations in IL6, proMMP1 and MMP3 release under specific D1-like and D2-like receptor activation in different concentrations. Here, the incubation time of 24h might have been too short, or optimized drug treatment prior to surgery might also lead to the observed reduced effects.
Cell migration and also motility under specific D1-like and D2-like receptor activation was highly age-dependent on both RA and OA patients. Younger patients (<75 years) showed a significant increase of cell migration and motility,

whereas older patients (>75 years) showed a significant decrease of migration an motility after D1-like and D2-like receptor activation, both in different concentrations and compared to the respective unstimulated control. Although effects were very similar between RASF and OASF, effects were stronger in RASF.

In this study we further investigated the dopamine system in RASF and showed, that dopamine has significant age-dependent effects on cell migration and motility, crucial character traits of RASF.

As DR activation has opposite effects on cell migration and motility according to the patient's age, the dopaminergic system in RA should be further investigated in order to enable adequate treatment.

Chapter 7

Summary (German)

Die rheumatoide Arthritis (RA) gehört zu den systemischen Autoimmunerkrankungen, die zu chronischer Entzündung der Gelenke führt. Typischerweise sind die kleinen Gelenke der Hände betroffen, allerdings sind auch extraartikuläre Manifestationen häufig. Etwa 1% der Weltbevölkerung ist betroffen, ein Großteil davon befindet sich bei Diagnosestellung noch im erwerbsfähigen Alter.
Auch wenn sich die Behandlungsmöglichkeiten in den letzten Jahren deutlich verbessert haben, ist eine Heilung nach wie vor nicht möglich. Im Rahmen der Entzündung in den Gelenken kommt es typischerweise zur Hyperproliferation der Synovia. Die synovialen Fibroblasten (SF) dringen dabei in perisynoviale Gelenkstrukturen ein und zerstören so Knorpel und Knochen, was letztendlich zur Zerstörung des Gelenks führt. Zudem sezernieren sie pro- und antiinflammatorische Zytokine und nehmen so aktiv am entzündlichen Geschehen im Gelenk teil.
In vorangegangenen Studien konnte gezeigt werden, dass auch SF ein intrinsisches Dopaminsystem besitzen und alle Dopaminrezeptorsubtypen auf SF exprimiert werden. Im Vergleich zu SF von Arthrosepatienten (OA), zeigte sich bei SF von RA Patienten eine deutlich erhöhte Expression von allen Dopaminrezeptor (DR) Subtypen.
In den durchgeführten Experimenten konnten wir zeigen, dass D1DR, D2DR, D3DR und D5DR auch in der Invasionszone von RA Patienten exprimiert werden. D1DR, D2DR und D5DR wurden dabei in der Invasionszone stärker exprimiert als im restlichen Synovium. Dies lässt eine Beteiligung der DR am agressiven Verhalten der RASF vermuten. Des Weiteren waren alle untersuchten DR im "lining layer", also in der obersten Schicht der Gelenkhaut mit direkten Kontakt zur Synovialflüssigkeit, stärker exprimiert als in den darunter liegenden Schichten. Dies traf sowohl für RA als auch für OA zu. Im Gegensatz zu vorherigen Veröffentlichungen konnten wir keine relevanten Veränderun-

gen in der Ausschüttung von IL6, MMP3 und proMMP1 nach spezifischer DR-Stimulation feststellen. Möglicherweise reichten die 24 Stunden Inkubationszeit hier nicht aus, oder die verbesserte medikamentöse Therapie der Patienten vor der notwendigen Operation führte hierzu.
Die untersuchte Zellmigration und -motilität von RASF und OASF unter jeweils spezifischer DR-Stimulation und in verschiedenen Konzentrationen führte zur einem signifikanten altersabhängigen Effekt. Während SF von jüngeren Patienten (<75 Jahre) eine signifikant erhöhte Migration und Motilität aufwiesen, zeigten ältere Patienten (>75Jahre) eine signifikant reduzierte Zellmigration und -motilität im Vergleich zur unstimulierten Kontrolle. Dieser Effekt zeigte sich sowohl bei RA als auch bei OA Patienten, allerdings ausgeprägter bei RA Patienten.
Die gesteigerte Zellmigration und -invasion von RASF ist ein zentrales, pathognomonisches Element der rheumatoiden Arthritis, und wir konnten hier zeigen, dass Dopamin dies signifikant beeinflusst. Da ein ausgeprägter altersabhänger Effekt nachgewiesen werden konnte, sollte dies näher untersucht werden um Patienten mit rheumatoider Arthritis eine altersgemäße, adäquate Therapie zukommen lassen zu können.

Chapter 8

Appendix

8.1 Abbreviations

Table 8.1 Abbreviations

AChR	Acetylcholine receptor
ACPA	Anti-citrullinated protein antibody
ACR	American College of Rheumatology
bDMARD	Biological DMARD
BH4	Tetrahydrobiopterine
bsDMARD	Biosimilar DMARD
cAMP	Cyclic adenosine monophosphate
CD	Cluster of differentiation
CIA	Collagen-induced arthritis
CNS	Central nervous system
CREB	cAMP responsive element binding protein
CRP	C-reactive protein
csDMARD	Conventional synthetic DMARD
DA	Dopamine
DAT	Dopamine transporter
DIP	Distal interphalangeal joint
DMARD	Disease-modifying anti-rheumatic drugs
DR	Dopamine receptor
ELISA	Enzyme-linked ImmunoSorbent Assay
EULAR	European Alliance of Associations for Rheumatology
GCPR	G-Protein coupled receptor
GTP	Guanosintriphosphate
IL	Interleukin
JRA	Juvenile Rheumatoid Arthritis
MAPK	Mitogen activated Protein Kinase
MCP	Metacarpal joint
MMP	Matrix metalloproteinases
MTX	Methotrexate
OA	Osteoarthritis
PBS	Phosphate buffered saline
PIP	Proximal interphalangeal joint
PKC	Proteinkinase
PNS	Peripheral nervous system
RA	Rheumatoid Arthritis
RF	Rheumatoid factor
RLS	Restless Legs Syndrome
ROS	Reactive oxygen species
SCID	Severe combined immunodeficiency
tsDMARD	Targeted synthetic DMARD
TGF	Transforming growth factor
TH	Tyrosine hydroxylase
TNF	Tumor necrosis factor
VMAT	Vesicular monoamine transporter

List of Figures

List of Tables

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Chapter 9

Declaration

Ehrenwörtliche Erklärung

„Hiermit erkläre ich, dass ich die vorliegende Arbeit selbständig und ohne unzulässige Hilfe oder Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe. Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten oder nichtveröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht. Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“ niedergelegt sind, eingehalten sowie ethische, datenschutzrechtliche und tierschutzrechtliche Grundsätze befolgt. Ich versichere, dass Dritte von mir weder unmittelbar noch mittelbar geldwerte Leistungen für Arbeiten erhalten haben, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen, und dass die vorgelegte Arbeit weder im Inland noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde zum Zweck einer Promotion oder eines anderen Prüfungsverfahrens vorgelegt wurde. Alles aus anderen Quellen und von anderen Personen übernommene Material, das in der Arbeit verwendet wurde oder auf das direkt Bezug genommen wird, wurde als solches kenntlich gemacht. Insbesondere wurden alle Personen genannt, die direkt und indirekt an der Entstehung der vorliegenden Arbeit beteiligt waren. Mit der Überprüfung meiner Arbeit durch eine Plagiatserkennungssoftware bzw. ein internetbasiertes Softwareprogramm erkläre ich mich einverstanden.“

Ort, Datum

Unterschrift

Chapter 10

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