CHAPTER 1

General introduction
Idiopathic pulmonary fibrosis (IPF) is a chronic and progressive disease that is characterized by pathological deposition of extracellular matrix (ECM) in the lung interstitium [1]. IPF is therefore classified as an interstitial lung disease (ILD). Roughly 25% of all ILD cases are IPF, which can be distinguished from other forms of ILD by the presence of usual interstitial pneumonia and the exclusion of other diagnoses (e.g., connective tissue disease, chronic hypersensitivity pneumonitis, and asbestosis) [2]. As IPF progresses, functional lung tissue is gradually replaced by ECM which significantly hampers gas exchange (i.e., influx of O₂ and efflux of CO₂), thereby leading to progressive breathlessness and, eventually, respiratory failure. Because of incomplete global epidemiological data, studies point toward an incidence rate for IPF ranging from 2 to 30 cases per 100,000 person-years and a prevalence ranging from 10 to 60 cases per 100,000 people [3]. In the worst-case scenario, these figures translate to an approximate population prevalence of 200,000 in the United States, 300,000 in Europe, and 960,000 in East Asia. IPF has also been shown to be more common in older populations. In fact, the prevalence of IPF among adults over the age of 65 has been determined to be ~500 cases per 100,000 people [4]. Furthermore, patients suffering from IPF have a poor prognosis; studies indicate a median survival time of approximately 3-5 years from the time of diagnosis [3].

**PATHOGENESIS**

After being injured, lungs normally display the ability for self-repair, which refers to the gradual replacement of dead and damaged tissue by healthy tissue [5]. This self-repair program is comprised of four distinct phases and includes a clotting/coagulation phase, an inflammation phase, a fibroblast recruitment/proliferation phase, and a remodeling phase [6]. In the first phase, following injury, epithelial and endothelial cells rapidly secrete cytokines to initiate an anti-fibrinolytic coagulation cascade, which leads to the formation of a provisional ECM composed of, among other proteins, fibrin and fibronectin [7]. During the ensuing inflammation phase, blood vessels at the site of injury dilate and become more permeable, thus enabling the recruitment of inflammatory cells (e.g., neutrophils, macrophages, lymphocytes, and eosinophils). These cells orchestrate the removal of injured cells and debris from the affected tissue [8]. In the third phase, fibroblasts are recruited to the site of injury where they start to proliferate – a process induced by elevated cytokine secretion during the inflammation phase. Upon activation, these fibroblasts turn into myofibroblasts, which are the main producers of ECM proteins, such as collagens [9]. Finally, in the last phase, myofibroblasts cause contraction of affected tissue, and epithelial as well as endothelial cells proliferate and migrate over the newly produced ECM. Afterwards, redundant myofibroblasts become senescent or undergo apoptosis [10].

In some cases, this self-repair mechanism becomes disrupted. As a consequence, the injured site can develop a profibrotic environment that causes myofibroblasts to produce vast quantities of ECM proteins (predominantly collagen type 1) [3]. The exact cause of this disruption is unknown, but several risk factors have been identified. For example, previous research has shown cigarette smoking is strongly associated with IPF, especially when people smoked more than 20 pack-years [11]. Another risk factor includes occupational exposure to metal, wood, coal, and livestock-related dusts. Inhalation of these dusts may contribute to the development of IPF [12]. (Chronic) viral infections have been associated with the development and exacerbation of IPF as well, though their contribution to the pathogenesis remains unclear [13]. Finally, genetics are also involved in the pathogenesis, albeit to a relatively low extent (< 5% of the cases). Various genes have been associated with the development of IPF, but it is not fully known how such genes affect the pathogenesis [14]. To summarize, the pathogenesis of IPF remains poorly understood which makes the development of an effective and safe drug challenging.

**TREATMENTS**

To date, limited treatments exist for patients suffering from IPF. Available treatments are classified as either pharmacological or non-pharmacological interventions. Approved pharmacological interventions include the use of pirfenidone (Esbriet®) and nintedanib (Ofev®). Though pirfenidone and nintedanib do not actually cure IPF, randomized and placebo-controlled phase 3 trials have demonstrated both drugs slow the decline in forced vital capacity by approximately 50% over the course of 1 year [15,16]. However, the therapeutic mechanism of pirfenidone remains unclear; although the drug has been shown to have antifibrotic, anti-inflammatory, and anti-oxidant properties in animal models of pulmonary fibrosis [17]. The mechanism-of-action of nintedanib has been more comprehensively documented; nintedanib is a strong inhibitor of receptor tyrosine kinases (e.g., platelet-derived growth factor, fibroblast growth factor, and vascular endothelial growth factor receptors) which can lead to attenuation of fibrosis-related processes [17]. Nevertheless, these drugs can cause serious side effects, such as gastro-intestinal bleeding, diarrhea, and liver toxicity [1].
Aside from prescribing drugs, clinicians are also recommended to provide non-pharmacological interventions, such as oxygen supplementation, pulmonary rehabilitation, and lung transplantation [14,18]. Oxygen supplementation, for example, has been shown to reduce exertional dyspnea (i.e., shortness of breath during exercise) and thereby improve exercise tolerance [19]. Patients may also benefit from pulmonary rehabilitation programs, which involve regular aerobic exercise, education, and psychosocial support, because it improves the quality of life [20]. Lung transplantation may be considered for patients with moderate to severe disease because it significantly improves the survival rate in comparison to patients who do not undergo transplantation surgery [14,18]. Due to a shortage of donor lungs, however, not all patients are considered for lung transplantations; only those with the highest survival probability are selected [21]. Pharmacological and non-pharmacological interventions are thus clearly limited. As a result, there remains an urgent unmet medical need for more effective and safer drugs to treat IPF.

To disrupt the expression of fibrosis-related genes for therapeutic purposes or to unravel the pathogenesis of IPF, scientists can exploit RNA interference – a powerful endogenous mechanism that can be induced with small interfering RNA (siRNA) which leads to specific messenger RNA (mRNA) and protein knockdown [22]. Therefore, the main goal of this thesis was to explore the use of siRNA within the context of pulmonary fibrosis. First, chapter 2 critically evaluates animal studies to determine whether pulmonary administration is a feasible approach to achieve site-specific delivery of siRNA in the lungs. Although this approach is promising, several key challenges were identified, such as the lacking correlation between in vitro and in vivo experiments. To address this issue, we assessed whether precision-cut tissue slices could be used as a model to study the effects of siRNA. To that end, chapter 3 describes the development of a transfection method for lung (and kidney) slices, using self-deliverable (Accell) siRNA, which successfully induced mRNA knockdown. With a focus on lung slices, chapter 4 reports a further extension of the previously developed transfection method by also demonstrating knockdown of proteins that are involved in fibrogenesis. In addition, preliminary results indicated that knockdown of these proteins also affected fibrogenesis. To address concerns with respect to the viability of lung slices, chapter 5 delineates the effect of incubator oxygen concentration (20 vs. 80% O₂) on cell death, anti-oxidant transcription, acute inflammation, and cell proliferation. Culturing lung slices at 20% O₂ dramatically improved their viability. Thereafter, chapter 6 presents the application of our transfection method to evaluate effects upon knockdown of heat shock protein 47 (HSP47) in fibrogenic lung slices. HSP47 was selected as it is involved in collagen maturation [23]. Surprisingly, collagen maturation in lung slices was not diminished upon knockdown of HSP47. Finally, chapter 7 summarizes key findings, critically evaluates them, and presents directions for future research.
BIBLIOGRAPHY


