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**Title:** Mesenchymal stromal cell treatment for COPD : experimental and clinical studies  
**Issue Date:** 2017-09-07
SUMMARY AND GENERAL DISCUSSION

CHAPTER 7
SUMMARY AND GENERAL DISCUSSION

The aim of this thesis was to investigate airway epithelial injury and potential repair mechanisms by mesenchymal stromal cells (MSCs), in the context of chronic obstructive pulmonary disease (COPD). COPD can develop in susceptible individuals following chronic exposure to inhaled noxious compounds, such as cigarette smoke, which is the main risk factor for COPD. The presenting symptoms of chronic cough and dyspnea relate to the pathogenic processes underlying the disease: enhanced inflammatory responses, airway epithelial remodelling, mucus hypersecretion and impaired clearance, and destruction of lung parenchyma [1]. COPD significantly contributes to disease burden and health care costs, and has a high impact on an individual patients' quality of life. It has remained the third leading cause of death worldwide over the past decade [2], and despite widespread availability of therapies that relieve COPD-related symptoms, there are no treatments that halt disease progression or moreover: cure COPD.

In search for such a curative treatment for COPD, disease mechanisms need to be unravelled to find potential modifiable targets. Based on current knowledge, obvious directions for novel therapies include those targeting the major denominators of COPD: inflammation and tissue destruction. In this light, cell therapy is a candidate new treatment, as cell-based therapies may modify inflammation and regenerate destructed tissue [3,4]. Cell therapy refers to administration of living cells, and its clinical application has shown promising results in a variety of diseases, including Graft-versus-host-Disease (GvHD) [5]. As a cell source, particular interest has arisen in the use of MSCs. The popularity of MSCs is partly explained by the fact that these cells can be relatively easily obtained from e.g. the bone marrow or adipose tissue, their high expansion rates ex vivo, as well as the lack of HLA-DR expression which helps to prevent recipient (host) immune responses upon administration of allogeneic cells. MSCs constitute a heterogeneous population of cells with properties of both stem and progenitor cells, and immunomodulatory and regenerative properties as established in preclinical studies in vitro and in vivo [6]. MSCs were shown to reduce inflammation, apoptosis and restore alveolar damage in animal models of emphysema [7-9]. However, it remains unclear whether similar effects on repair and inflammation can be obtained in humans and if so, how MSCs exert these effects.

This thesis’ aim was therefore to investigate the effects of MSCs and MSC-secreted factors on airway epithelial repair following injury, in the context of COPD. The presented studies describe a novel in vitro model of airway epithelial injury, and assess the effect of MSCs both in vitro as well as in patients with COPD. The main findings are briefly summarized, followed by a discussion of the presented studies in a broader perspective.
SUMMARY OF MAIN FINDINGS

The first part of this thesis investigated airway epithelial repair, focussing on the effects of cigarette smoke and MSC-secreted factors on wound healing in vitro.

Cigarette smoke (CS), the major risk factor associated with COPD was found to delay epithelial wound closure and modulate innate immune responses in primary bronchial epithelial cells cultured at the air-liquid interface (ALI-PBEC). Effects of CS on basal cells (BCs) included selectively induced expression of the BC-derived antimicrobial protein *RNase 7* following CS exposure, and impairment of migration and spreading of BCs into the wounded area. The effects of CS on wound repair and immunity were found to be mediated in part by oxidative stress, and via EGFR and ERK1/2 activation (Chapter 2).

We next demonstrated that airway epithelial repair can be enhanced by MSC-secreted factors, in particular when MSCs are pre-conditioned with pro-inflammatory cytokines, which significantly induced the expression of several growth factors in MSCs. The underlying mechanisms include activation of ERK1/2 signaling in airway epithelial cells, predominantly via direct activation of EGFR and transactivation, and induction of airway epithelial EGFR-ligand expression (Chapter 3).

In the second part of this thesis we focussed on clinical implementation of autologous MSCs in patients with COPD.

We first demonstrated that autologous bone marrow-derived MSCs (BM-MSCs) from patients with severe to very severe COPD are phenotypically and functionally comparable to MSCs from age-matched controls. Functional assays included response to pro-inflammatory stimuli and effects on airway epithelial cells. Based on minor differences with MSCs from controls, it is speculated that MSCs from COPD-donors share characteristics with aged MSCs, including decreased adipocyte differentiation and altered oxidative stress responses (Chapter 4).

These studies supported the role of autologous MSCs for clinical use in COPD. In the clinical trial, we demonstrated the safety of autologous BM-MSCs administration in patients with severe to very severe COPD, without MSC-related effects on clinical parameters and on most of the analysed tissue parameters. However, increased expression of the endothelial cell marker CD31 and increased numbers of CD3+ T-cells in alveolar walls were observed. It is tempting to ascribe these exciting observations to MSC-related effects, but the study design did not allow for such conclusions as a control group was lacking (Chapter 5).
Although MSC administration in patients with COPD appears to be safe, the results from three independently conducted clinical trials have not been able to demonstrate clinically relevant effects. This is in contrast with the results from in vitro and in vivo models of lung epithelial and endothelial injury, where MSCs were shown to restore lung architecture and decrease inflammation. The discrepancy between these preclinical data and clinical results might relate to differences in optimization of treatment regimens and to a lack of proper outcome parameters (Chapter 6).

The discussion will start by highlighting models to study lung development and repair, including our perspective on the importance of primary epithelial cultures to investigate repair in the human lung in the context of COPD. Next, our view on the directions of future studies with MSCs and how to improve our understanding of MSC efficacy and behaviour in humans will be discussed. Finally, perspectives to optimize MSC-based cell products and alternative strategies using MSCs in regenerative medicine are evaluated, and a general conclusion and outlook into future COPD management in light of regenerative medicine is presented.

SECTION I MODELS OF AIRWAY EPITHELIAL INJURY

There is an urgent need to better understand repair mechanisms in the lung, to find new treatments and ultimately hopefully a cure for patients with COPD. In this regard, increased knowledge of lung development and physiological maintenance of the airway epithelium will be of great importance, as well as insight in airway epithelial responses to injury relevant to the topic of COPD. Appreciation of physiological processes will elucidate whether its dysregulation evolves into pathology, and will potentially provide useful tools for new intervention strategies to prevent or even reverse the development of lung diseases.

Insight in lung development and physiological maintenance of the airway epithelium has increased considerably over the past decade. Several studies have elucidated the role of transcription factors, growth factors and other signaling molecules, including Wnt and retinoic acid, during lung organ maturation, for instance via reconstructing the process of branching morphogenesis combined with transgenic mouse technologies in vivo or using matrigel cultures of lung endoderm in vitro (as reviewed in [10]). Information about stem and progenitor populations, their location and differentiation during lung development in the murine lung was obtained using “lineage tracing”, a technique in which a transgenic (labelled) construct is inserted in the cell of interest, allowing tracing of these cells and their progeny [11]. This technique has also proven useful for investigating airway epithelial responses following injury in vivo, and thus contributed to our understanding of the progenitor function of basal cells in airway epithelial repair [12]. Since targeted genetic
manipulations are not feasible in humans, in humans mutations in mitochondrial DNA were used to identify and trace cells of interest and their progeny [13]. This way, clonal expansion of single airway epithelial progenitor cells was studied and progenitor cells were identified that maintain the upper airways in humans, and effects of aging and smoking on the heterogeneity of progenitor cell populations were demonstrated. For future research, this model can be used to compare the composition of epithelial progenitor cell populations between healthy subjects, healthy smokers and smokers with COPD and this may shed light on the hypothesis that COPD originates from alterations in basal cell biology [14], advance our understanding why not all smokers develop COPD and provide targets for early treatment interventions.

To gain insight in COPD pathogenesis and potential new treatment targets, it is common to use animal models of emphysema. Induction of emphysema is achieved by exposure to cigarette smoke (either alone or combined with lipopolysaccharide) or intratracheal instillation of proteolytic enzymes like elastase to induce pulmonary emphysema [15]. Alternatives include pharmacological induction of emphysema by treatment with vascular endothelial growth factor (VEGF)-receptor blockers or the use of transgenic mice [16,17]. Using animal models, therapeutic effects of several agents, including e.g. retinoic acid, growth factors (hepatocyte growth factor (HGF), VEGF) and MSC-based cell therapies have been investigated [18-20]. However, it is clear that the rodent lung does not accurately represent human biology, reflected also by the so far limited clinical success of therapies with retinoic acid [21] or MSCs [22], which underlines the importance of models more representative of the human situation.

In this light, the use of in vitro cell cultures of human cells obtained from the airway (primary bronchial epithelial cells (PBEC), peripheral lung (alveolar type 2 (AT2) cells) or pulmonary endothelium (lung-derived human primary microvascular endothelial cells (HPMVEC)) are increasingly relevant, preferably using cultures of primary cells. Primary cells represent a cell population that is present in the human body and more accurately reflect in vivo conditions compared to cell lines that are immortalized or tumor-derived. The benefit of cell culture studies relates to the possibility of controlled manipulation of specific cellular functions and processes, and simplification of complex interactions that are difficult to study in vivo. Using this model, processes essential to wound repair were elucidated, including spreading, migration and proliferation, as well as the interactions with extracellular matrix (ECM) and role of signaling molecules (reviewed in [23]). Frequently used in vitro injury and repair models include chemical, mechanical (e.g. scratch wounds) or electrical disruption of epithelial layers. By combining these models with exposure to a variety of airborne substances, such as allergens, air pollution, microbial pathogens, the effect of these environmental exposures on the repair process can be studied [24-26]. Apart from investigating barrier responses to injury, in vitro models have been used to detect differences between COPD patients and controls regarding wound closure rates, immunologic responses following injury,
tight junction formation and levels of Wnt expression [27-31], demonstrating its value to detect potential mechanisms involved in COPD pathogenesis.

Given the causative role of airborne toxicants to COPD development, it is essential to investigate airway epithelial cell responses to airborne toxicants, particularly to cigarette smoke (CS). Commonly, CS effects are tested using CS-extract (CSE): a filtered solution containing the noxious compounds from CS in suspension. CSE was shown to cause a loss of barrier integrity and differential effects on wound closure depending on the CSE-concentration were shown [29,32], but standardization of CSE preparation is difficult and the application to the cells does not reflect the in vivo situation. In Chapter 2 we presented a more physiological model: PBEC cultured at the air-liquid interface were exposed to whole CS at the air-exposed side. Our laboratory has gained long-term experience using PBEC cultured at the air-liquid interface [33,34], reflecting in vivo conditions of the airway epithelium [35]. As demonstrated, this model allows combining CS-exposure with mechanical and chemical injury, enabling detailed investigation of repair and inflammatory responses. Options to extend the information derived from this model are numerous. For example: a detailed study of differentiation of cells, restoration of cell junctions and tracking of cells of interest using time lapse movies. This adds to knowledge derived using CSE or non-primary cells [29,36,37]. Furthermore, CRISPR-Cas9 based gene editing tools can be used to identify key mediators in repair. Besides, it would be highly relevant to set-up cocultures of AT2 with HPMVEC to mimic the gas-exchange unit to increase our understanding of emphysema development and obtain new targets for therapy. Apart from the simplicity of in vitro models, it also offers a physiologically relevant alternative to animal studies, in compliance with a universal aim to reduce the number of animals needed for biomedical research. This also accounts for more novel in vitro culture systems, including organoids, microfluidic lung-on-a-chip and lung tissue slices. It is desirable that future studies on repair will increasingly make use of such in vitro systems.

SECTION II OPTIMIZATION OF MSC-BASED TREATMENT REGIMENS

Prior to publication of data derived from the clinical trial described in chapter 5, one clinical trial investigating efficacy of intravenous administration of MSCs in patients with COPD had been published. This phase II trial conducted in 62 patients with moderate to severe COPD demonstrated safety of MSC administration, but was unable to show clinical improvement of pulmonary function parameters or quality of life [22]. The lack of clinical effects of MSC treatment has not discouraged research in this field, and the results presented in our clinical trial (Chapter 5), i.e. an increase of CD31 expression and changes in inflammatory cell numbers in alveolar walls, in fact support efforts to further explore the potential of MSCs to induce airway epithelial repair.
To do this, there are some obvious questions that require attention, for instance clarification of the optimal cell source, dosage, timing and route of administration during the course of the disease, as also argued by others [39]. Thus far, clinical trials that were conducted to investigate MSC efficacy have used heterogeneous protocols (even within organ systems and diseases), and were predominantly designed as safety studies without a control group [40]. This has hampered optimization of MSC-treatment protocols. As we have come to the point where MSC administration is considered safe [41], further clinical trials with expedient comparisons of the mentioned items using matched controls is a logical first step. This also asks for measurable outcome parameters to assess MSC-related effects, which in case of COPD trials typically include functional responses (e.g. pulmonary function testing, performance, quality of life), laboratory parameters and quantitative imaging. However, these parameters might not be as modifiable as we had hoped, as demonstrated by the negative phase II trial by Weiss et al [22]. This is potentially due to delayed effects of MSCs on these parameters, requiring prolonged treatment programs and/or follow-up. To circumvent this, parameters that may precede clinical improvement should be included, such as parameters of pulmonary inflammation and repair on tissue (similar to our approach as described in Chapter 5), as well as composition of inflammatory cells in sputum and bronchoalveolar lavage fluid (BALF), including cytokine concentrations. Adding bronchial and peripheral biopsies would allow analysis of potential effects on alveolar and endothelial structures, inflammatory infiltrates and airway epithelial remodelling, but requires invasive procedures to obtain tissue. Ideally, less invasive methods or biomarkers are needed to assess effects, e.g. in blood. It is postulated that although alterations in these parameters might not directly translate in clinical improvement, they might be early indicators that the progressive course of COPD can be amended, and changes in such ‘basic’ parameters might be more practical endpoints during optimization of MSC-treatment protocols in future clinical trials.

Routes of MSC administration to consider in COPD treatment include intratracheal instillation and intravenous administration. Intratracheal instillation was found to modify pulmonary inflammation and disease severity in bronchopulmonary dysplasia in new-borns [42]. Whether a similar response can still be expected in the adult lung needs to be elucidated, but is suggested by the decreased levels of C-reactive protein following intrabronchial MSC administration in COPD patients [43]. Intravenous administration on the other hand is probably relevant to target the periphery of the lung and the vascular component in particular, as our own data tentatively indicated responsiveness of the endothelium (Chapter 5). If either effect is indeed present, it seems reasonable to personalize the optimal route of MSC-administration, implying intravenous administration if COPD is characterized by emphysema and tissue destruction, and intratracheal administration when airway obstruction and chronic bronchitis predominate; or a combination of both.
Other items that need attention to optimize MSC treatment include MSC-host interactions and use of allogeneic versus autologous cells. The efficacy of MSCs appears to depend at least in part on MSC-host interactions, probably related to local inflammation, retention and survival of MSCs within subjects [5]. Increasing our understanding of MSC-host interactions is therefore important but it is also difficult, since MSCs do not express unique markers and labelling of MSCs in humans is restricted, but alternative (non-nuclear) labelling techniques are currently explored and may become available in the near future for use in humans as well [44,45]. Meanwhile, alternative models are needed to assess MSC-host interactions, and the use of ex vivo lung perfusion (EVLP) models can be of benefit in this respect. EVLP employs a laboratory set-up to preserve lungs that are unsuitable for lung transplantation, with the aim to maintain these lungs under physiological circumstances (albeit outside the human body) using a perfusion circuit and protective lung ventilation [46]. Its value in MSC-research was shown in a model of acute lung injury [47,48]. Using EVLP, labelled MSCs can be infused and tracked to gain insight in MSC homing, retention and survival in the lung. The information can be extended to assess MSCs’ responses to COPD-related tissue destruction or inflammation, following for instance instillation of proteolytic enzymes (e.g., elastase) or even exposure of EVLP to cigarette smoke via the ventilator. It should be taken into account that the clinical translation of this model is limited by a lack of interactions with other organs and systemic responses and the relatively short preservation time of the model (approx. 7 days), which does not reflect the chronic course of COPD. EVLP-donor characteristics should also be considered, notably the fact that the lungs were rejected for transplantation implying some degree of organ dysfunction. However, this could become an advantage if lungs were rejected due to smoking-related disease, which would allow comparisons with ‘healthy’ donor lungs.

In clinical trials, both allogeneic and autologous MSCs are used. The advantage of allogeneic MSCs relates to their potential use as an ‘off-the-shelve’ therapeutic making them suitable for acute diseases, but they carry the risk of evoking alloimmune responses [49,50]. Autologous MSCs are unlikely to elicit such immune responses but are thought to display age- and disease related impairments [51], and their application is logistically more challenging and time-consuming. So far, based on the data presented in Chapter 4 we consider treatment with autologous MSCs suitable in patients with advanced COPD, taking into account that the results should not be generalized to current smokers since acute effects of CS on bone marrow cells were not assessed. As both allogeneic and autologous MSCs have their advantages and disadvantages, it seems reasonable to compare their efficacy in clinical trials, including immune monitoring testing to assess safety of allogeneic MSCs, as has been suggested by others as well [49].
SECTION III INCREASING THE THERAPEUTIC POTENTIAL OF MSC-BASED CELL THERAPIES

Apart from creating uniformity in study protocols for MSC administration, there is a need to create uniformity of MSC cultures themselves to improve interpretability of (pre-) clinical data. MSC cultures are heterogeneous due to a lack of MSC-specific markers and use of different culture protocols, contributing to functional variation between cell products (discussed in [52,53]). Minimal potency requirements of MSC-based cell products are still undefined, as it is unknown which assays best predict the potency of MSCs [54] and to what extent this potency in vitro translates to increased potential in vivo [52]. Besides, the desired potency profile of MSCs may differ between diseases. Proposed evaluation of MSCs’ potency for use in COPD patients includes proliferation and migration potential, mRNA expression of several growth factors and cytokines in response to COPD-relevant inflammatory mediators, and wound repair potential, as we have presented in Chapter 4. For future investigations, interactions with immune cells should also be included, for instance T-cell proliferation assays and MSC-induced polarization of macrophages. Besides, interactions of MSCs with endothelial cells should be further investigated. This approach may contribute to the identification of superior cell products that are more effective at targeting COPD-related inflammation and tissue damage.

Continuing on, the next step should be to determine characteristics of these ‘superior’ cell products with respect to the composition of its subpopulations (as proposed in [53]). For instance, although still a matter of debate subpopulations of MSCs were shown to differentiate into lineages other than the mesenchyme, implying pluripotency of a fraction of MSCs (reviewed in [55]), and there is some evidence that links heterogeneity of MSCs to efficacy in vivo [56]. In potential, identification of ‘superior’ subpopulations can increase knowledge on their relative contribution to the cell products’ potency and could contribute to development of a more purified cell product with increased clinical potential, or identify vectors to treat specific diseases. Such an approach using a subpopulation of MSCs, referred to as “multi-lineage differentiating stress enduring” (MUSE) cells [57], is in fact being developed by a Japanese-based company called Clio (www.clio-inc.com), and clinical trials from this company are awaited to demonstrate whether this approach should be carried forward.

Another approach to increase the therapeutic potential of MSC-based cell products encompasses pre-conditioning of MSCs during culture, for example by using pro-inflammatory mediators, growth factors or hypoxic culture conditions (reviewed in [58]). In vivo, compared to control-cultured MSCs, interferon-γ stimulated MSCs ameliorated colitis in mice [59], and MSCs cultured under hypoxic conditions protected against bleomycin-induced pulmonary fibrosis in mice [60], supporting the idea that preconditioning of MSCs increases their therapeutic potential. We observed increased expression of several growth factors and signaling molecules
following stimulation of MSCs with pro-inflammatory cytokines (Chapter 3), coinciding with increased regenerative potential of MSCs in vitro. However, manipulating MSCs prior to clinical administration warrants caution as potential adverse effects might be underestimated in animal models, and long term effects are unclear. To the best of our knowledge, this approach has thus far not resulted in clinical trials, except for one clinical trial that is currently recruiting patients with COPD to assess effects of hypoxia-cultured MSCs compared to placebo treatment (NCT01849159, to be completed in June 2017).

Similarly, genetic engineering of MSCs may increase their therapeutic potential, as for instance shown for MSCs overexpressing angiopoietin 1 (ANGPT1) or Interleukin (IL)-10 in mice models of acute respiratory distress syndrome [61,62]. However, genetic engineering or targeted gene addition usually involves transfection of cells using viruses as vectors, which limits the applicability in humans but does not make it impossible, as demonstrated by the use of gene therapy in patients with inherited primary immunodeficiency diseases [63]. The potential hazards of this virus-based approach can be bypassed in the future by using other gene editing tools, such as CRISPR-Cas9 based gene editing. Alternatively, MSCs are investigated as delivery vectors of therapeutic agents including nano-particles, suicide gene/enzyme prodrug systems, or oncolytic viruses, predominantly in the field of cancer research [64], but this can be applied to the field of regenerative medicine as well. However, to our knowledge no clinical data on either approach are available to date.

SECTION IV ALTERNATIVE STRATEGIES TO TARGET TISSUE REPAIR

Clinical trials in COPD using MSCs have investigated effects of bone marrow-derived MSCs via administration of whole live cells. However, within the field of regenerative medicine other approaches to tissue repair are being developed, including exploration of the potential to activate endogenous lung progenitor cells including lung-resident MSCs, ex vivo tissue engineering and the use of induced pluripotent stem cells. These topics will briefly be addressed to generate a sense of the position of MSC-based therapies within this area of research.

MSCs reside in many different tissues other than the bone marrow, including the lung [65,66]. Lung-resident MSCs (LR-MSCs) possess distinct phenotypical and functional characteristics when compared to bone marrow-derived MSCs, including higher expression of lung-related signaling genes such as FOXF1 and SFRP1 (involved in Wnt signaling) [67]. LR-MSCs are suggested to form part of the lung stem cell niche, and although their specific contribution to the niche is still largely uncharacterized, animal studies indicate that they support epithelial stem cell growth and differentiation (as reviewed in [39]). As repair mechanisms in the lung are thought to be deficient
in COPD, it would be interesting to investigate endogenous repair by LR-MSCs, including the possibility of activating LR-MSCs to enhance repair, or the potential of LR-MSCs to activate key signaling pathways in other local progenitor cells, including activation of Wnt signaling which was found to attenuate experimental emphysema [68]. A comparison of LR-MSCs from COPD versus non-COPD controls will be relevant in this respect. Besides, lung-derived MSCs may be considered as a source for cell therapy: LR-MSCs can be obtained via bronchoscopy with BAL or peripheral biopsies and display high expansion rates in vitro, which are favourable properties for their potential use as cell therapy [65,66], and have a longer retention time in the lung compared to bone marrow-derived MSCs [69]. It is conceivable that increased retention might have beneficial effects on restoration of destructed lung tissue, especially in chronic diseases such as COPD, but this requires further investigation.

Another potential source of cell therapy is formed by induced pluripotent stem cells (iPSCs). iPSCs are derived from adult somatic cells (frequently skin fibroblasts), that are first reprogrammed towards cells with embryonic stem cell properties, and from this state can differentiate into theoretically any cell type [70,71], including MSCs (iMSCs) [72] and airway and alveolar epithelial (progenitor) cells [73]. Research in this field has provided important information on pathways that regulate lung development and epithelial cell differentiation, including bone morphogenetic protein (BMP), Wnt, fibroblast growth factor (FGF) and nodal signaling pathways [10,73,74]. Following exploration of pathways and signaling requirements to reprogram iPSCs towards pulmonary epithelium, it is conceivable that iPSCs can be used to investigate how modifications of reprogramming protocols influences epithelial cell development, which might increase our understanding of development of respiratory diseases. Besides, detailed knowledge on how these pathways are regulated should in theory enable us to selectively activate epithelial progenitor cells and the stem cell niche in vivo, with the ultimate aim to induce or enhance activity of endogenous stem cells for tissue repair. However, safety margins of such interventions are probably small, as demonstrated for instance by the relation of Wnt and Notch signaling with occurrence of fibrosis, cystic formation and cancer [75-77].

Apart from induction of endogenous repair or administration of cell therapies, an area of attention in the field of regenerative medicine consists of tissue engineering, i.e. the construction of functioning lungs ex vivo using a synthetic structure or decellularized lung as a scaffold, which is subsequently coated with cells that cover the scaffold and differentiate into pulmonary epithelium [78-80]. In potential, combining this approach with iPSCs as a patient-specific cell source will reduce the number of donor lungs needed and simultaneously eliminate problems related with graft rejection [71]. Bone marrow and adipose tissue-derived MSCs were also shown to adhere to scaffolds and differentiate towards lung epithelial phenotypes [81], supportive of their potential use as a stem cell source in tissue engineering, although functionality of this approach still needs
to be demonstrated. As an intermediate step before whole-organ engineering, implantation of smaller scaffolds at sites of severe tissue destruction might improve tissue structure as demonstrated in sheep: endobrochial placement of a scaffold covered with MSCs at sites pre-treated with elastase resulted in local tissue regeneration and improvement of supporting matrix [82]. Similarly, application of sheets coated with adipose tissue-derived cells in rats having had lung volume reduction surgery for emphysema increased alveolar and vascular regeneration and improved gas exchange and exercise tolerance [83]. Although largely unexplored, such alternative approaches to deliver MSCs (or other stem cells) at sites of tissue destruction should be considered in humans as well. In this light, alternative tissue engineering approaches like a ‘lung-on-a-chip’, a micro-physiological system that replicates the functional gas-exchange unit of the living human lung, also bears the potential to contribute to gas-exchange in destructed parts of lung tissue, provided that such a device can be integrated into local tissue.

FUTURE DIRECTIONS AND GENERAL CONCLUSION

After reading the discussion, the impression may remain that the field of cell therapy in the context of COPD is characterized by questions rather than answers, and is still in its infancy. However, although we acknowledge the many uncertainties for the future of MSC-based cell therapies for COPD, we feel that this area of research has progressed towards its puberty, with a growing body of evidence on the mode of action and safety of MSC-treatments in several diseases. Nevertheless, it does challenge us with new questions.

It is likely, that different strategies for regenerative medicine are needed at various stages of the disease (see Figure 1). In limited disease, when airway epithelial progenitors and stem cell niches are still responsive to key signaling pathways, these pathways could be activated by a pharmacological approach in order to restart or enhance local tissue maintenance programs. Upon further disease progression, restoration of tissue architecture via exogenously administered MSCs or other stem/progenitor cell populations could be considered. Ideally, these stem cells are integrated within the local stem cell niche and will orchestrate restoration of pulmonary epithelium from here. In severe end-stage disease, when it has become impossible for stem cells to adhere to local tissue due to severe destruction, engineered scaffolds could be implanted in the lung or in more severe cases the whole lungs may be replaced by ex vivo engineered lungs to restore pulmonary function. Maturation of this area of research will reveal whether this direction for the future therapy of destructive lung diseases such as COPD is realistic.
In conclusion, the studies described in this thesis have provided novel insight into airway epithelial repair mechanisms and their modulation by cigarette smoke, and insight into mesenchymal stromal cell treatment of COPD. We have shown that inflammatory mediators present in the lungs of patients with COPD increase the regenerative potential of MSCs, and that MSCs from patients with severe COPD can be safely used as a cell-based therapy to treat these patients. Many questions remain regarding route of administration, dosage and timing of MSCs administration in COPD. Useful outcome parameters to assess MSC-mediated effects on lung tissue are largely undetermined, and we propose to include analysis of effects on endothelial and inflammatory cells in future clinical trials. The use of ALI-PBEC and alveolar epithelial cell cultures and ex vivo lung perfusion models will help to advance our understanding of the potential of MSCs in pulmonary diseases. Parallel developments in other areas of regenerative medicine, including those related to induced pluripotent stem cells and ex vivo organ engineering, will synergistically advance the much awaited therapeutic arsenal that is needed to restore pulmonary function in COPD.

Figure 1. Proposed approach to implement (future) regenerative strategies to restore destructive lung disease. Various stages of tissue destruction require different approaches to accomplish tissue repair, related to the presence and responsiveness of endogenous cell populations. In lack of a population of endogenous stem cells that is able to regenerate damaged tissue, exogenous stem or progenitor cells can be administrated to support or even restore the local stem cell niche. When tissue architecture is damaged severely, engineered scaffolds or lungs can be used to restore organ function.
## LIST OF ABBREVIATIONS

<table>
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<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>ALI</td>
<td>air-liquid interface cultured</td>
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<tr>
<td>ANGPT1</td>
<td>angiopoietin 1</td>
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<td>AT2</td>
<td>alveolar type 2 cells</td>
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<tr>
<td>BAL(F)</td>
<td>bronchoalveolar lavage (fluid)</td>
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<td>BC</td>
<td>basal cell</td>
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<td>BM-MSC</td>
<td>bone marrow-derived MSC</td>
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<tr>
<td>BMP</td>
<td>bone morphogenetic protein</td>
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<td>CS(E)</td>
<td>cigarette smoke (extract)</td>
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<td>CD</td>
<td>cluster of differentiation</td>
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<tr>
<td>COPD</td>
<td>chronic obstructive pulmonary disease</td>
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<tr>
<td>CRISPR/Cas 9</td>
<td>clustered regularly interspaced short palindromic repeats/Cas 9</td>
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<td>ECM</td>
<td>extracellular matrix</td>
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<td>EGFR</td>
<td>epidermal growth factor receptor</td>
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<td>EVLP</td>
<td>ex vivo lung perfusion</td>
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<td>ERK1/2</td>
<td>extracellular signal-regulated kinase 1/2</td>
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<td>FGF</td>
<td>fibroblast growth factor</td>
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<td>FOXF1</td>
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<td>GvHD</td>
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<td>HGF</td>
<td>hepatocyte growth factor</td>
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<tr>
<td>HLA-DR</td>
<td>human leukocyte antigen D related</td>
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<td>human primary microvascular endothelial cells</td>
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<td>IL</td>
<td>interleukin</td>
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<td>iPSC</td>
<td>induced pluripotent stem cell</td>
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<td>LR-MSC</td>
<td>lung resident MSC</td>
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<td>MSC</td>
<td>mesenchymal stromal cell</td>
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<td>MUSE</td>
<td>multi-lineage differentiating stress enduring</td>
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<td>PBEC</td>
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