Stem cell-based therapy and regenerative approaches to diseases of the respiratory system

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Introduction: Despite treatment advances in many diseases of the respiratory system, outcome remains poor.

Sources of data: This systematic review (PubMed and Ovid) ‘analyses stem cell (SC)-based therapy and regenerative medicine (RM) approaches as potential novel strategies for diseases of the respiratory system. Current preclinical research and ongoing clinical trials are presented and their potential clinical impact and routine application discussed.

Areas of agreement: These approaches may represent a promising alternative therapy for otherwise irreversible respiratory diseases. Several experimental and initial clinical data now exist.

Areas of controversy: Type of SC, limits of tissue engineering, route of delivery, cell behaviour (differentiation, growth, co-stimulation or immunomodulation) and interaction with the human microenvironment upon implantation.


Areas timely for developing research: The potential capacity of SC-based therapy and RM should be carefully investigated before their translation into clinical practice.

Keywords: regenerative medicine/tissue engineering/SC-based therapy/respiratory diseases

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Introduction

Despite recent diagnostic and therapeutic advances, respiratory disease is the most commonly reported long-term illness in children and the third in adults. Only in the UK, more people die from respiratory disease than from ischaemic heart disease with about 70.7 respiratory disease-related deaths per 100 000 population or one in five people, a death rate that is almost double the European average. Therefore, an alternative therapeutic option is needed and desired. Regenerative medicine (RM) is an emerging interdisciplinary field of research and clinical applications focused on the repair, replacement or regeneration of cells, tissues or organs to restore impaired function resulting from any cause, including congenital defects, disease, trauma and ageing. It uses a combination of several approaches that moves beyond traditional transplantation and replacement therapies. These include, but are not limited to, the use of soluble molecules, gene therapy, SC transplantation, tissue engineering (TE) and the reprogramming of cell and tissue types.\(^1\)

While SC-based therapy (cell therapy) and its combination with natural or synthetic scaffolds to replace organs and tissues (TE) (Fig. 1) have been established as a clinical standard of care for conditions, such as haematopoietic SC transplants for leukaemia and epithelial SC-based treatments for burns and corneal disorders. Their applications to patients afflicted by incurable respiratory diseases and disabling conditions are still at preclinical or early clinical stages and have not yet been shown to have a clear-cut advantage over existing therapies. In this review, the different approaches of RM that are likely to play a pivotal role in the clinical treatment of diseases of the respiratory system are described and the currently performed clinical trials presented.

![Fig. 1](image_url) Regenerative medicine for respiratory diseases includes stem-cell therapy and TE.
Stem cells

Stem cells (SCs) are the primary descendants of every living organism, and our lives rely on persisting SCs to regenerate organs and tissues that are injured or lost. They reside in specific regions of tissues and organs (SC niches) in a dormant form, and have two unique characteristics: (a) ‘self re-newal’: they can divide and give rise to more SCs of the same kind and (b) under proper conditions, they can ‘mature or ‘differentiate’ into specialized cells performing a specific function, such as in the skin, muscle or blood.2

From the embryonic period and on, SCs go through different developmental cycles and are considered ‘omnipotent’ (capable to differentiate into every cell type or complete organism), ‘pluripotent’ (can differentiate into any cell derived from the three germ layers, ecto-, meso- and endoderm), ‘multipotent’ (lineage restricted, e.g. as mesenchymal or haematopoietic) or ‘oligopotent’ and ‘unipotent’ SCs (few or one cell type, respectively).

There are many different types of SCs that may be used for RM, including (i) ‘embryonic SCs’ (ESCs): they exist only at the earliest stages of development, are pluripotent with therefore the highest therapeutic potential; their application and research is, however, ethically and scientifically controversial. (ii) ‘Tissue-specific’ SCs: reside in various different tissues and organs of high (e.g. blood, skin, gut, etc.) and less (e.g. brain) regenerative potential and are usually referred to as ‘adult’ or ‘somatic’ SCs. They are already somewhat specialized (e.g. mesenchymal or haematopoietic SC—MSCs, HSCs) and because they produce only a limited number of cell types which are considered ‘multipotent’. They are the most commonly used cells for SC-based therapy. (iii) ‘Induced pluripotent SCs (iPS cells): cells with properties similar to ESCs that have been engineered from specialized cells such as skin cells; although they might open new therapeutical possibilities, recent evidence exists that they may result in genomic abnormalities and therefore their clinical application can only be realized under extreme caution.3 (iv) ‘Progenitor cells’ (PCs): these cells are described as oligopotent and tend, like SCs, to differentiate into specific types of cells (e.g. angioblasts or endothelial PCs, etc.), and are already more specific than an SC and obligated to differentiate into its a defined phenotype. The unlimited versus limited self-renewal capacity (both in vitro and in vivo) and the multipotency versus uni- or oligopotency of SCs, iPSCs and PCs, respectively, are the main differences between these two cell lines (Table 1).

Whatever the strategy—SC therapy or TE—the question of the source of the cells is of paramount importance. Since bone marrow-derived
SCs and PCs can be easily mobilized and recruited to boost a site-specific regeneration and/or to reseed and (micro) vascularize an acellular biomaterial implanted \textit{in vivo} to help it to become a living cell substitute, we hypothesize that these cells will play a fundamental role in the near future for the treatment of patients with respiratory diseases. However, not only autologous but also allogeneic cells have great potential for clinical use. The hypoimmunogenicity of MSCs for instance allows for wide allogeneic application.

**SCs and progenitor cells of the respiratory system**

Residual adult SCs have been detected in nearly all different types of tissues and organs, including the respiratory system. These local cells are called resident (or SC-niches), and are located along the basal layer and submucosal glands of the large airway and the alveolar epithelial surface of the distal airway (Fig. 2), and responsible for tissue regeneration and repair. Putative airway PCs have been so far identified inside pulmonary neuroendocrine cell rests, at the bronchoalveolar junction and alveolar epithelial surface. It is believed that they participate in complex cell-dependent and cell independent lung tissue regeneration for instance via a Fgf10-dependent pathway.\(^4,5\) Beside their

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**Table 1 Differences between stem cells and progenitors cells.**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>SCs</th>
<th>iPSCs</th>
<th>Progenitor cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethical issues</td>
<td>Embryonic SCs(^a)</td>
<td>Disputable</td>
<td>No ethical issues</td>
</tr>
<tr>
<td></td>
<td>Adult SCs(^b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Self-renewal \textit{in vivo}</td>
<td>Unlimited</td>
<td>Unlimited</td>
<td>Limited</td>
</tr>
<tr>
<td>Self-renewal \textit{in vitro}</td>
<td>Unlimited</td>
<td>Unlimited</td>
<td>Limited</td>
</tr>
<tr>
<td>Maintenance of self-renewal</td>
<td>Yes</td>
<td>Likely yes</td>
<td>No</td>
</tr>
<tr>
<td>Potentiality</td>
<td>Pluripotent/multipotent</td>
<td>Pluripotent</td>
<td>Usually unipotent, sometimes oligopotent</td>
</tr>
<tr>
<td>Population</td>
<td>Reaches maximum numbers of cells before differentiation</td>
<td>Not determinated</td>
<td>Does not reach maximal population</td>
</tr>
<tr>
<td>Significant risk of teratoma</td>
<td>SC type-dependent risk</td>
<td>Significant risk</td>
<td>No teratoma risk</td>
</tr>
<tr>
<td>Immunological properties</td>
<td>Cell type-dependent MHC expression level</td>
<td>Normal level of MHC I and II when derived from adult cells</td>
<td>Normal developed immunogenicity (MHC I, II)</td>
</tr>
</tbody>
</table>

SCs, stem cells; MHC, major histocompatibility complex; iPSCs, induced pluripotent SCs.

\(^a\)Serious.

\(^b\)No or marginal ethical issues.
regenerative potential, residual SCs might also influence the immune surveillance and induce immunomodulatory effects, particularly MSCs. Due to their key role in different regenerative processes, SCs are interesting candidates for scientists to target with gene transfer or boosting (or accelerating) factors. The potential role of the recently discovered lung cancer SCs for tumour progression and development as well the evidence that local PCs may shift into cancer SCs is under active investigation.5,6

Unfortunately, the self-renewal properties of resident SCs decline with age, and, therefore, non-resident or exogenous SCs/PCs are attractive cell source alternatives. ‘Exogenous SCs’, like ESCs, MSCs, HSCs or iPSCs can be isolated from different tissue types (e.g. bone marrow, fat, etc.),7–9 in vitro cultured, expanded, differentiated and applied to a patient. The most popular SC type in respiratory diseases is the mesenchymal SCs. Their interest relates to their multipotency, easy culture and their ‘hypoimmunogenicity’.10 This is due to the lack of

Fig. 2 Detected stem and progenitor cell-niches along the respiratory tract. Potential stem and progenitor cell-niches along the respiratory tract (in mouse). In the trachea and large airways, progenitor and SCs have been identified in basal layer and submucosal glands (in mice, in contrast to human, submucosal glands are only located in the proximal trachea). Kulchitsky progenitor cells (neuroendocrine progenitor cells) are highly associated with the origin of lung cancer. Potential progenitor cells, both in human and mouse, have been so far identified inside pulmonary neuroendocrine cell rests (neuroepithelial body; NEB), at bronchoalveolar junction and alveolar epithelial surface, such as bronchoalveolar SCs (BASCs), Clara cell secretory protein (CCSP) expressing cells or calcitonin gene-related peptide (CGRP) expressing cells. However, the definite determination as a resident stem or progenitor cell is still challenging and identification still not standardised.
MHC-II and low MHC-I expression, their ability to prevent and suppress T cell responses indirectly via the modulation of dendritic cells or indirectly via the inhibition of NK proliferation and cytotoxicity or T cell function (both CD8+ and CD4+). Beside this, they are ‘ethically’ neutral. MSCs are highly capable to differentiate into other specific mesenchymal tissue related-cell types, such as cartilage, bone, bone marrow stroma, muscle, fat, ligament or dermis. Moreover, MSCs have an immunomodulatory impact on the microenvironment. Due to their capacity to produce and secrete a variety of paracrine factors and bioactive macromolecules, they became a key player in lung tissue injuries. MSCs can be obtained via bone marrow aspiration or biopsies, followed by an isolation procedure to make them serviceable and then directly applied or past cell culture. Concerning cell delivery, several experimental animal and initial clinical trials suggest that systemic (venous) injection, administration via the trachea (either endoscopically or transectaneously) or site specific are feasible routes of delivery and sufficient to guarantee cell viability and reach the target location.

Although several positive effects have been described for MSCs, we do not entirely understand cell-promoted associated pathways or induced processes. One can speculate that the level of engraftment of donor MSCs is in most diseased conditions very low and most effects of cell administration might be related to several paracrine pathways.

The question of how MSCs behave and how they differentiate in variable milieu has to be investigated more precisely even if the in vitro differentiation of MSCs is well established. There is still a lack of exact knowledge concerning in vivo cell differentiation and therefore an incautious clinic application of MSCs should be strictly avoided.

**Signalling molecules and delivery of genes**

Signalling molecules, proteins or factors have a significant influence in cell migration, differentiation and guidance of cellular ingrowth to the environmental milieu. The philosophy behind this technology is to activate and support the self-healing potential of the organism. SCs for instance should be mobilized from their niches, migrate to target locations and start to proliferate or differentiate. One uses the already existing mechanisms and pathways but potentiates or accelerates their operating sequence. The delivery of molecules and genes could likewise be applied to enhance and modify graft and scaffold characteristics to improve cell engraftment for example. There are different ways to deliver a specific molecule or a gene to an organism, such as recombinant DNA incorporated in a vector, which is transduced to the cell, or administration of cells that express this specific signalling molecule/
gene or even via nanotechnology. However, the target cells that are potentially affected by the signalling molecule/or gene should be capable of responding to this signalling. Therefore, an exact knowledge about cell activation and response is absolutely necessary before moving toward bedside application.

**Tissue engineering**

The application of scaffolds or matrices to grow new tissues or organs from isolated cells, tissue or synthetic compounds is the basic principle in TE. Although major achievements have focused on tissues constructed using thin sheets of cells, such as bladder, skin and arteries, increasing evidence now exists that TE can be used to replace seriously diseased respiratory tissues and organs, such as the larynx, trachea and lungs. Basically, three different components are necessary to design a functional tissue engineered tissue or organ: (i) a natural or synthetic scaffold or matrix, (ii) autologous or allogeneic cells and (iii) a bioreactor. The required scaffold or matrix should be bioactive, capable to host the seeded cells, and not immunogenic, toxic, carcinogenic or teratogenic. Ideally, the matrix mimics the target tissue environment and maintains the required tissue-specific mechanical properties. It has to be biocompatible and the biomaterials may be natural (i.e. demineralized bone and decellularized cartilage matrix, intestinal mucosa, collagen, sponge, etc.) or artificial (i.e. polymers, metals, polypropylene, poly-l-lactic acid mesh) in origin. Unlike scaffolds made of synthetic materials, natural matrices, consisting mainly of extracellular matrix, are degraded by cellular enzymatic activity, release growth factors and peptides that stimulate tissue remodelling and angiogenesis (neoformation of vessels). Therefore, the choice of the optimal scaffold influences cell proliferation, differentiation, migration and polarity significantly. Matrices can be further manipulated by adding specific signalling molecules or peptides to enhance cell engraftment or differentiation.

**Stem-cell and tissue engineering for respiratory diseases**

**Nose and pharynx**

Nasal cavity or pharynx-related disorder are predominately benign and self-limiting, such as rhinitis or pharyngitis. For patients suffering from chronic inflammatory and/or allergic conditions in this area, the immunomodulatory capacity of SCs might be interesting but has been so far only rudimentarily investigated. Regarding nose and pharynx
malignancies, conventional treatment includes chemo-, radiotherapy and surgical intervention. This last option would eventually need reconstructive tissue to restore the surgically created defect. TE is a potential solution to resolve the lack of available tissue; Lendlein et al.\textsuperscript{.23} suggested the use of elastic shape polymers in the hypopharynx region as potential biomedical grafts. Zhang et al. reported satisfying results in patients undergoing total hypopharyngectomy treated with an artificial biological material—acellular dermal matrix (alloderm)—to reconstruct surgical wounds.\textsuperscript{.24} They observed survival of the biomaterials, no signs of fistula and a complete covering of the treated area by growing epithelium postoperatively. However, a wide number of experimental studies need to be performed and evaluated before a routine clinical application will be feasible.

\textbf{Larynx}

The standard care for many patients suffering from irreversible laryngeal disease remains total laryngectomy with the consequently loss of speech and difficulties to breathe. So far only, two documented laryngeal allotransplants have been performed on humans but both required life-long immunosuppression and this might explain why this procedure has not gained worldwide acceptance. The availability of natural or synthetic substitutes displaying equivalent anatomical, physiological and biomechanical properties of normal human larynxes would be therefore extremely welcome and would provide the right complex architecture and dynamics for normal voice production and sphincter action. Especially because it has been demonstrated that most of the larynx can be removed with the preservation of one muscle-nerve-joint unit and, as a consequence, without the need of neuromuscular activity regeneration. TE for laryngeal replacement is still in its childhood but advances have been made. We very recently produced bioengineered human larynxes using a decellularization process and are confident that their \textit{in vivo} implantation will provide precise anatomical reconstitution and native cartilaginous support for functional partial hemilaryngectomy replacement in terms of airway, voice and swallowing without the need of immunosuppression.\textsuperscript{25}

\textbf{Trachea}

The trachea can be affected by a broad variety of disorders and usually surgical intervention is curative. Unfortunately, the resectable length is restricted to around 6 cm in adults and 1/3 of tracheas length in
children, and reconstructive tissue is limited. In 2008, we performed the first transplantation of a bioengineered trachea in humans. Since then, eight other patients have been treated with this technique, and among them three cancer patients. The lessons learned so far are that even if decellularized, human tracheal matrices contain residual proangiogenic factors (e.g. vascular endothelial growth factor) that, once implanted into humans, induce neovascularization within only a few days, that epithelial respiratory cells reseed the internal tracheal lumen usually two months after transplant and that after 30 months follow-up, no stem-cell related disorders (tumours, etc.) were observed. We also learned that, in humans, PCs can be mobilized and recruited with pharmacological intervention to boost the generation of the implanted grafts, and that by using specific growth factors, undifferentiated MSc can be differentiated into viable chondrocytes as soon as 1 week after transplantation. This TE technology relies on a human donation of a trachea but other more nanomedicine-driven synthetic biomaterials are under active investigation.

**Lungs**

Lung-related diseases, such as chronic obstructive pulmonary disease (COPD), pulmonary hypertension (PH), cystic fibrosis (CF) and lung cancer, represent a leading cause for mortality and morbidity worldwide. Despite recent advances, the overall outcome remains poor and the only available therapy remains lung transplantation. Unfortunately, given the shortage of human donors and the need of life-long immunosuppression, novel strategies are more than needed. Here is an overview on the actual status in the field (Tables 2 and 3):

**SC-based therapies**

*Chronic obstructive pulmonary disease*

Several animal models have mimicked COPD conditions and evaluate cell-based therapeutic strategies. Induced tissue damage (via tobacco smoke or degradative enzymes) improved using bone marrow SCs (intra-bone marrow transplantation) with decreased emphysematous structural changes. Others investigated allogeneic MSCs injected systemically in papain-induced emphysema and detected an attenuation of airspace enlargement and alveolar apoptosis. Despite these significant changes, only a low level of donor cell was detected. The notable improvement could therefore rather be explained by the cells bioactive and paracrine capacity. Based on these findings, first clinical trials have been designed. There is currently a multicentre, double-blind, placebo-controlled phase II trial conducted by Osiris, Inc. (NCT00683722)
Table 2  Previous and present clinical trials.

<table>
<thead>
<tr>
<th>Current clinical trials</th>
<th>Previous clinical trials</th>
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<tbody>
<tr>
<td>Endothelial Progenitor Cells and Pulmonary Idiopathic Arterial Hypertension (NCT00551408). Unidad de Investigacion Clinica en Medicina SC, Monterrey, Mexico. Principal investigator: Carlos J. Sanchez Diaz, MD. <strong>Objectives:</strong> to demonstrate if the number of EPCs is increased in a Mexican population of patients with pulmonary arterial hypertension—Recruiting</td>
<td>Safety and Efficacy Study of Transplantation of EPCs to Treat Idiopathic Pulmonary Arterial Hypertension (NCT00257413). The First Affiliated Hospital, College of Medicine, Zhejiang University, China. Principal investigator: Junzhu Chen, MD. <strong>Objectives:</strong> to investigate the feasibility, safety and initial clinical outcome of intravenous infusion of autologous EPCs in patients with idiopathic pulmonary arterial hypertension (IPAH)—Recruiting</td>
</tr>
<tr>
<td>Pulmonary Hypertension: Assessment of Cell Therapy (PHACE-T) (NCT00469027). St. Michael's Hospital, Toronto. Principal investigators: Michael Ward, MD; David Langleben, MD; Sir Mortimer B. Davis, Jewish General Hospital, Montreal. <strong>Objectives:</strong> to establish the safety of autologous progenitor cell–based gene therapy of heNOS in patients with severe pulmonary arterial hypertension refractory to conventional treatment—Recruiting</td>
<td>Safety and Feasibility of Autologous Endothelial Progenitor Cells Transplantation in Patients With Idiopathic Pulmonary Arterial Hypertension (NCT00641836). The First Affiliated Hospital, College of Medicine, Zhejiang University, China. Principal investigator: Junzhu Chen, MD. <strong>Objectives:</strong> 1-year follow-up to investigate the safety and efficacy of autologous EPCs injection in patients with IPAH.</td>
</tr>
<tr>
<td>Safety Study of Cell Therapy to Treat Chronic Obstructive Pulmonary Disease (COPD-01) (NCT01110252). Laboratório de Genética Humana e Terapia Celular, Assis, São Paulo, Brazil. Principal investigator: João T Ribeiro-Paes. <strong>Objectives:</strong> to determine whether the cell therapy with bone marrow mononuclear cells is safe in the treatment of COPD, specifically the pulmonary emphysema—Recruiting</td>
<td>Safety Study of Cell Therapy to Treat Chronic Obstructive Pulmonary Disease (COPD) (NCT00683722). Multicenter designed study. Responsible Party: Osiris Therapeutics, Inc. (Robin Flannery). <strong>Objectives:</strong> to establish the safety and efficacy of multiple administrations of PROCHYMAL™ (human adult stem cells) in subjects with moderate to severe COPD—Active, not recruiting</td>
</tr>
<tr>
<td>PROCHYMAL™ (Human Adult Stem Cells) for the Treatment of Moderate to Severe Chronic Obstructive Pulmonary Disease (COPD) (NCT00683722). Multicenter designed study. Responsible Party: Osiris Therapeutics, Inc. (Robin Flannery). <strong>Objectives:</strong> to establish the safety and efficacy of multiple administrations of PROCHYMAL™ (human adult stem cells) in subjects with moderate to severe COPD—Active, not recruiting</td>
<td>Detection of Circulating Endothelial Progenitors Cells (EPCs) in Non-small Cell Lung Cancer (NSCLC) (CBNPC:PCE; NCT00826683). CHU Limoges, France. Principal Investigator: Boris Melloni, Professor. <strong>Objectives:</strong> to identify EPCs in peripheral blood from patients with NSCLC in comparison with COPD, an inflammatory disease—Recruiting</td>
</tr>
<tr>
<td></td>
<td>Safety and Efficacy Study of Transplantation of EPCs to Treat Idiopathic Pulmonary Arterial Hypertension (NCT00372346). The First Affiliated Hospital, College of Medicine, Zhejiang University, China. Principal investigator: Junzhu Chen, MD. <strong>Objectives:</strong> investigate the feasibility, safety and initial clinical outcome of intravenous infusion of autologous EPCs in patients with idiopathic pulmonary arterial hypertension (IPAH)—Recruiting</td>
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</table>

Patients suffering from moderate-to-severe COPD are treated with *ex vivo* cultured adult human mesenchymal SCs. One of the intermediate results is the lack of side-effects or infusional toxicity during cell administration. Beside this, a significant reduction of circulating C-reactive protein, usually increased in COPD patients, has been detected as well as an improved trend in the quality of life. Another active trial investigates the effect of systemically administrated (peripheral vein) bone marrow mononuclear cells (NCT01110252) (Table 2). The 12-month follow-up revealed a stable clinical condition, which can be referred to a significant change in the natural progression of this
Table 3: Stem cell therapy and RM approaches in different animal models.

<table>
<thead>
<tr>
<th>Animal model</th>
<th>Cell therapy</th>
<th>Findings</th>
<th>Underlying pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary hypertension</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Induced via MCT (^{17–19,44})</td>
<td>Allogeneic, autologous MSCs, BMD PCs, eNOS, PCycS and AM gene transfer into PCs</td>
<td>Decreased PVR, RVH, PAP, improved acetylcholin response, RV function</td>
<td>Paracrine effect, VEGF, engraftment</td>
</tr>
<tr>
<td>Pulmonary artery ligation (^{14})</td>
<td>MSCs</td>
<td>Decreased PVR, PAP alter of protein expression level</td>
<td>Paracrine, cell dependent</td>
</tr>
<tr>
<td>Abdominal aorta to inferior vena cava (^{16})</td>
<td>CGRP gene transfer into EPCs</td>
<td>Decreased PAP, PVR</td>
<td>Incorporation and local bioactive effect</td>
</tr>
<tr>
<td>Hypoxia (^{45})</td>
<td>Inhibition SDF-1/CXCR4</td>
<td>Decreased of PAP, RVH, vascular cell proliferation, reduced vascular remodelling</td>
<td>Decreasing PC recruitment to pulmonary vasculature</td>
</tr>
<tr>
<td>Chronic/acute inflammation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Induced via LPS i.t., (E. coli)</td>
<td>MSCs, Ang 1 gene transfer to MSCs</td>
<td>Reduced lung injury, proinflammatory (TNF-alpha, MIP-2) cytokines and inflammatory cells</td>
<td>Paracrine, bioactive capacity</td>
</tr>
<tr>
<td>LPS i.p. (^{31,32,36})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bleomycin (i.t.) (^{33–35})</td>
<td>MSCs (BMD, human umbilical cord derived), transferred KGF</td>
<td>Reduced lung tissue injury, fibrosis, inflammatory cytokines</td>
<td>Cell engraftment and paracrine effects</td>
</tr>
<tr>
<td>Lung transplant (^{53})</td>
<td>EPCs</td>
<td>Vascular repair</td>
<td>Endothelial integrity in vessels, cell incorporation</td>
</tr>
<tr>
<td>PDOC (^{54})</td>
<td>ESCs, MSCs</td>
<td>Self-recovering of airways epithelium</td>
<td>No adequate model to study SCs capability</td>
</tr>
<tr>
<td>Cecal ligation and puncture (^{47})</td>
<td>BMSCs</td>
<td>Improved survival and organ function</td>
<td>Bioactive effects, prostaglandin E2-related IL-10</td>
</tr>
<tr>
<td>Bronchopulmonary dysplasia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Induced via Chronic hyperoxia (^{10})</td>
<td>MSCs</td>
<td>Improved survival, PAP, exercise tolerance attenuated alveolar, lung vascular injury</td>
<td>Paracrine effects, AEC2-specific marker surfactant protein C</td>
</tr>
<tr>
<td>Asthma</td>
<td></td>
<td></td>
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<tr>
<td>Induced via Ovalbumin (^{21})</td>
<td>MSCs</td>
<td>Reduced hyperresponsiveness of airway</td>
<td>Paracrine effect, inhibition of Th2 TGF-beta increase</td>
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<tr>
<td></td>
<td></td>
<td>Reduced asthma-specific pathological changes</td>
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</tbody>
</table>

MCT, monocrotaline; BMD, bone marrow derived; PCycS, prostacyclin synthase gene; AM, adrenomedullin; PVR, pulmonary vascular resistance; RVH, right ventricular hypertrophy; PAP, pulmonary artery pressure; VEGF, vascular endothelial growth factor; CGRP, calcitonin gene-related peptide; SDF-1, stromal-derived factor-1 and its receptor CXCR4; LPS, lipopolysaccharides; i.t., intratracheal; i.p., intraperitoneal; Ang 1, angiopoietin-1; MIP-2, macrophage inflammatory protein 2; KGF, keratinocyte growth factor; PDOC, polidocanol; AEC2, type II alveolar epithelial cell; Th2, T helper cell; TGF-beta, transforming growth factor.
disease, and amelioration in the quality of life. The so far obtained findings need verification with longer follow-up times.

**Acute lung injury**

Despite improvements in supportive care, current data report that the mortality rate is still around 40%. A variety of animal models showed the significant impact of MSCs in acute lung injury (ALI) conditions induced via lipopolysaccharide (LPS), bleomycin or hyperoxia exposure.\(^{30-36}\) In all of these studies, MSCs demonstrated their efficiency to reduce mortality, improve alveolar fluid clearance and/or change inflammatory reactions. Notably, the engraftment of donor MSCs was again, as mentioned before, minimal if at all existent, yet several paracrine pathways and effects have been described. Anti-inflammatory mediators like interleukin-10 (IL-10), IL-1ra, IL-13, angiopoietin-1, keratinocyte growth factor (KGF) increased after MSC treatment.\(^{34,36-39}\) Moreover, proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-\(\alpha\)) and macrophage inflammatory protein 2 (MIP-2) decreased significantly after cell administration.\(^{36}\) In summary, these data provide essential knowledge about the feasibility and efficiency of MSCs in ALI conditions, and may already claim the translation of stem-based therapy into clinical practice for ALI or severe acute respiratory distress syndrome.

**Pulmonary fibrosis**

Pulmonary fibrosis (PF) can be caused by several different inflammatory and non-inflammatory pathways. Its genesis might also be related to undefined autoimmune mechanisms. It is a chronic progressive severe disease with yet insufficient treatment and therefore associated with high mortality and morbidity rates. Several groups explored the underlying pathways and PF’s pathophysiology, with the most bountiful results being obtained by using bleomycin-induced animal models.\(^{33,34}\) Some utilized native MSCs,\(^{33}\) others administrated MSCs acting as a vehicle for gene therapy, transduced with genes such as angiopoietin-1 (Ang 1) to potentiate the protective effect for instance.\(^{36}\) Systemic MSC administration resulted in a reduction of inflammation and fibrosis formation. However, these effects were observed when MSCs were given shortly after inducing lung injury via bleomycin (4 hours after bleomycin) and disappeared when given on Day 7.\(^{33}\) Therefore, one can assume that MCS treatment would be less potent in patients with an already fixed and long-lasting PF. However, MSCs could be applied in early stages or as preventive strategy in conditions that are highly associated with the development of fibrosis, such as irradiation therapy. The significance of genetically modified and transduced SCs (i.e. keratinocyte growth factor, Ang-1) in the field of
fibrosis needs further experimental investigation.\textsuperscript{34,36} One specific SCs population, ‘circulating fibrocytes’, might play a key role both as an indicator for the severity of the fibrosis (also for other lung diseases) and its treatment.\textsuperscript{40} Due to their active participation in the progression of PF, they can embody the optimum vector to supply therapeutic agents to the region of fibrosis development. Currently there are no clinical trials.

\textbf{Cystic fibrosis}

Cystic fibrosis (CF) is a devastating autosomal recessive disorder caused by a mutation of the CF transmembrane conductance regulator protein (CFTR) encoding gene and leading to an abnormal ion transport via the chloride channel. These chloride channels can be found not only in lung tissue but also sinuses, pancreas, skin and gastrointestinal tract. In general, this defect results in a change of the viscosity and hydration of the fluids covering epithelial cells resulting in chronic respiratory infection and destruction of the airways tissue. In several mouse models, the engraftment of applied SCs was quite poor. Associated CFTR protein expression was likewise very low and therefore improvement of diseased conditions, if any, was marginal.\textsuperscript{41} Another approach in this field might be the \textit{in vitro} modification of SCs. Sueblinvong \textit{et al.} used cord blood-derived MSCs cultured with specialized airway growth media (+ specific growth factors) and transduced with vectors expressing human CFTR. Findings showed that MSCs expressed several phenotypic markers of airway epithelium (such as Clara cell secretory protein, cystic fibrosis transmembrane conductance regulator, surfactant protein C, thyroid transcription factor-1 mRNA, etc) yet their engraftment level maintained low.\textsuperscript{42} It seems that the systemic administration of SCs does not cause a significant engraftment and thus the replacement of the damaged epithelial cells fails to appear. However, their significance as an immunomodulator in terms of activating anti-inflammatory cytokines or inhibiting pro-inflammatory factors has so far not been determined. There are currently no clinical trials.

\textbf{Asthma}

Asthma is a chronic inflammatory condition of the lungs, characterized by reversible airflow obstruction, excessive responsiveness of the lungs to stimuli in the forms of infections, allergens, and environmental irritants. The bioactive capacity of MSCs to modulate the immunological response in organism has been demonstrated in a growing number of animal studies. Recently Goodwin \textit{et al.} suggest MSCs impact on allergic respiratory inflammation. This could recently be confirmed by Nemeth \textit{et al.}\textsuperscript{21,22} and will bring forward MSCs clinical application in patients with severe and chronic allergic conditions.
Pulmonary hypertension

Pulmonary hypertension (PH) is characterized by a proliferation of smooth muscle cells and deregulated control of endothelial cells in terms of dysfunction, apoptosis and profuse proliferation simultaneously. This remodeling process inside the pulmonary vessels results in an elevation of pulmonary vascular resistance, progressive pulmonary hypertension, right ventricular failure and finally to death. MSCs have already demonstrated their efficiency and reproducability as a therapeutic option in different animal models. Common models to investigate for effects and pathophysiology are monocrotaline- or hypoxia-induced PH. Clinical improvement were observed. Beside MSCs bioactive impact, in terms of vascular endothelial growth factors release, they are able to differentiate into epithelial cells and thus restore normal pulmonary function. In an animal study of monocrotaline, induced PH endothelial-like progenitor cells (ELPC) were utilized and transduced with human endothelial NO-synthase (eNOS). Findings of marked improvement in survival and restoring of microvasculature structure and function could be observed.

In our recently developed animal model of chronic thromboembolic pulmonary hypertension (CTEPH), MSC administration resulted not only in an improved clinical outcome and decreased inflammatory response, but also in a significant alteration of the expression level of proteins. These findings suggested once more MSCs immense bioactive capacity and their paracrine impact on inflammatory response. The model also demonstrated, as previously described, the homing effect on MSCs by damaged tissue. The multiplicity of these clear and distinct pre-clinical data makes them highly interesting for the clinical use. Two recent trials of autologous intravenous endothelial progenitor cell (EPC) infusion were enrolled at the Zhejiang University, Hangzhou, China (NCT00257413, NCT00641836) (Table 2). The purpose of these trials was to evaluate the effects of EPCs in term of feasibility, safety and initial clinical outcome in patients with idiopathic pulmonary arterial hypertension. The researchers observed an increased 6-min walk distance (common indicator to assess physical strength and verify diseases progression/improvement), ameliorated pulmonary artery pressure and vascular resistance. A third trial concerning autologous progenitor cells transplantation is currently performed at Zhejiang University (NCT00372346) and data can be expected soon. Another on-going two-centre trail raises hopes for clinical routine SC administration in pulmonary hypertension (NCT00469027); the purpose of this Phase I trial is to establish the safety of autologous progenitor cell-based gene therapy of heNOS in patients with severe pulmonary arterial hypertension (PAH) refractory to conventional
treatment. Initial findings showed no safety concerns or side effects after cell administration. Remarkably, the patients showed a significant reduction of the pulmonary vascular resistance. Furthermore, a clinical trail conducted in Monterrey, Nuevo Leon (Mexico) (NCT00551408) (Table 2) tries to answer the question of the number of EPCs in patients with PAH and thus their significance within this disease. Beside all these notable improvements and positive findings, some critical reports exist showing the contribution of bone marrow-derived cells to hypoxia-induced pulmonary arterial remodelling in animal models. More interestingly, it is suggested that SCs may contribute to systemic and pulmonary vascular remodelling in neonatal hypoxia. Therefore, their clinical administration has to be evaluated accurately before applying routinely.

Lung cancer
Due to the controversial discussion of uncontrolled SC differentiation, their use in cancer is fairly limited. Concerning the potential function of endogenous lung progenitor cells as cancer SCs, it is widely hypothesized that MSCs (both bone-marrow-derived or circulating) and EPCs might play a key role in the development of lung cancer or its promotion. Particularly their capacity of developing microvascularization and supportive stroma supply an environment for tumour progression. Beside their pluri- or multipotency, which might contribute essentially to the risk of tumour formation and progression, their relative apoptosis resistance and capacity to replicate for extended time periods can also rise the probability for tumour promotion and development. One has to be aware that the effects of administrated cells can differ significantly and this may be related to the variability in SCs from different sources (i.e. bone marrow-, umbilical blood- or tissue-derived SCs) and inter-donor variability (even from same sources). In addition to that, a potential tumour progression is dependent on several intrinsic and extrinsic mechanisms and factors (i.e. site of cell administration) and/or the necessity of in vitro culture. The in vitro manipulation/culture of SCs can result in completely contrary findings such as the in vivo generation of tumours or the reduction of tumour after ex vivo differentiation. A potential dedifferentiation or redifferentiation into unwanted cell type has been described and the clinical consequences remain unclear. Nevertheless, the tropism of SCs and thus their ability to home and migrate to region of tumour progression can be utilized for cell-based therapy against lung cancer. SCs that migrate automatically to tumour development can be genetically modified to release anti-tumour and pro-apoptotic factors what might induce tumour regression or reduction of progression. In contrast, EPCs associated with lung cancer might embody potential biomarkers in this
context. One clinical trial is currently investigating the role of EPCs in peripheral blood from patients with non-small cell lung cancer (NSCLC). The expected findings might help to highlight (NCT00826683) (Table 2) EPCs relevance as a predictive value in NSCLC.

**TE lungs**
During the last two decades, most achievements in TE have been with tissues constructed using thin sheets of cells, such as bladder, skin and arteries. Bioengineering of thicker tissues, such as muscle, heart and liver, has not been possible due to limited diffusion of nutrients and oxygen within the engineered tissue mass. This is because cells lying more than few (1–3) millimetres away from a source of nutrients and oxygen will eventually necrose. A significant bioengineering advance in airway replacement has been the utilization of decellularized three-dimensional scaffolds like the trachea and larynx. More recently, two different groups succeeded to experimentally (in young rats) decellularize lung scaffolds.\(^{50,51}\) They could reseed the scaffold with cells and show initial physiological and functional properties of these engineered lungs. Moreover, for a more specific and deep knowledge of the complex integrated organ-level, particularly in responses to inflammatory downstream signals, cytokines and cell-dependent pathways, Huh \textit{et al.}\(^{52}\) developed a biomimetic microsystem mimicking the alveolar-capillary interface. Even if we are far behind an entire functional tissue engineered lung, these technologies will bring forward its development and realization.

**Conclusions**
The advent of SC-based therapy and TE has the potential of revolutionizing treatment and therapies of patients with diseases of the respiratory system. By favouring repair and regeneration, SC therapies and TE may lead to strategies not only able to restore function of lost or damaged tissue or cells (e.g. alveolar-capillary membrane, pneumocytes, alveoli, etc.) but also bypassing the need for alloreplacement (e.g. larynx, trachea, lung), restoring thereby their function. However, the potential benefit to patients, especially those with end-stage respiratory diseases, should not be overstated and unrealistic timelines for their clinical application should not be given before a list of challenges including SC source and type, limitations of TE, in-depth knowledge of SCs in vivo behaviour and interaction or underlying pathways and mechanisms are fully elucidated. The use of SCs must be standardized and controlled in order to guarantee the highest level of safety for the
patients. Given, however, the enormous potential of regenerative medicine, it would be not unrealistic that these approaches will dramatically alter the practice of treating patients affected by failing respiratory tissues and organs.

References


33 Ortiz LA, Gambelli F, McBride C et al. Mesenchymal stem cell engraftment in lung is enhanced in response to bleomycin exposure and ameliorates its fibrotic effects. *Proc Natl Acad Sci USA* 2003;100:8407–11.


