The use of stem cells to repair the injured lung

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Introduction: The structure of the lung is complex, it contains at least 40 different cell types. The lung interacts with the outside environment and the circulatory system. These features make the lung particularly susceptible to injury and disease.

Areas of agreement: Stem cells with reparative properties can be found within the lung. Also, outside sources of stem cells can contribute to the repair of the injured lung. These include multipotent stem cells from the bone marrow and pluripotent stem cells derived from the early embryo or from adult cells, which are made to reverse to a pluripotent state by the addition of viral vectors or non-viral agents. For stem cells of outside sources to have a reparative function, the cells need to reach the injured lung, either by internal mobilization of stem cells from other parts of the body (e.g. bone marrow) or by administration of exogenous cell sources.

Areas of controversy: Much research is currently undertaken to define the mechanisms by which stem cells repair the injured tissue. These include the possibility of engraftment of exogenous cells or the release of growth factors from the cells to aid repair. There is not as yet a clear consensus as to the mechanisms of repair.

Current research and timelines: Interest is now focused on developing appropriate animal models to test the safety and efficacy of stem cell therapies and to understand the mechanisms by which stem cells undertake this task.

Keywords: resident stem cells/bone marrow stem cells/pluripotent embryonic stem cells/iPS cells

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Introduction

Charles Spurgeon (1834–1892), the leading preacher in Victorian England said: ‘Every generation needs regeneration’.

This is true for today’s day and age. There are millions of patients afflicted with chronic diseases for which there is neither a cure nor further available treatment options.

Structure of the lung

The structure of the lung is complex; it is packed with an elastic, dynamic structure and a large surface area for gas exchange (oxygen, O₂ and carbon dioxide-CO₂). The lung has double interactions: (i) with the outside environment and (ii) with the circulatory system. These features make the lung particularly susceptible to injury and disease. Repair of the lung is difficult due to the fact that the cells lining the air pipes (airways) do not self-renew as rapidly as in other organs, like the gut or the skin. Hence, resident stem cells of the respiratory tract, which in normal situations maintain airway integrity, can be overwhelmed in situations of lung injury or disease. In this situation, recruitment of stem cells from outside the respiratory system takes place (see later for details).

The lung comprises 23 generations of airways, a process that is made up by a series of branching airways, each smaller than the last, terminating in the gas exchange unit or alveolar sacs. These occupy a ‘large’ surface area. There are about 600 million alveoli in the human lungs and if stretched out, they would cover an entire tennis court.

At least 40 different cell types have been identified in the respiratory tract. The airways or windpipes are covered with a layer of cells, called epithelial cells, which forms the interface with the airspace. The windpipes or pulmonary tree is divided into distinct anatomical regions: it starts by a large airway, or cartilaginous windpipe, this is followed by smaller airways (termed bronchioles) down to the very last: the alveolar sacs (Fig. 1).

Tightly packed cells (called pseudostratified epithelium) line the larger airways. Similar lining is seen in the smaller airways. A special cell type is seen in this region, and it is called Clara cell, which plays an important role in detoxifying inhaled pollutants. The most outer/peripheral part of the airways is lined by two different types of cells: one (type 1) is very elongated and flat and covers about 95% of the surface area. Being long and flat presents little barrier for the diffusion of oxygen from the airways to the adjacent circulation (see diagram above) while
maintaining the integrity of the alveolar wall. The other type (Type 2) comprises only 5% of the alveolar surface area, even though the cells are numerous. The reason being that the cells are cuboidal and small (see Fig. 2). These cells are critical in maintaining integrity of the alveolar space by clearing the space from unwanted fluids. They also secrete a substance (surfactant protein) that prevents the airspace from collapsing.

The pulmonary circulation is composed of a continuous layer of flattened cells bathed on one side by blood and on the other side by interstitial fluid.

The space between the airspace and the blood vessels is called ‘interstitium’. Effectively is the ‘skeleton’ which holds all the various cells types together and on the right place and allows for plasticity during respiration. The ‘interstitium’ is composed of collagen/elastin or ‘scaffold’ material and harbours muscle and immune cells amongst others.

**Stem cells**

**Stem cells found in the lung**

This is an intensely researched field. No consensus has yet been reached as to the full characterization of these putative stem cells. However, it would appear that cells with ‘stem’ characteristics are found in all the different parts of the bronchial tree (airway pipes).
These cells maintain tissue integrity and are activated when lung repair is needed after tissue injury.\textsuperscript{1,2}

In the uppermost proximal part, where the large airways reside, the basal cells of the pseudostratified epithelium conform with most the characteristics of stem cells, in the smaller airways, the bronchioles, Clara cells are considered to be stem cells that maintain homeostasis, and in the peripheral parts of the lung, where the alveolar sacs are and where gas exchange takes place, the type 2 cells, precursors of Type 1 cells, are regarded as stem cells. All of these cells reside in specific tissue niches.

There are numerous, specific niches in the lung, where the stem cells reside. The niche in biology is defined as a particular structural area with specific cell/cell interactions, where physical factors influence cell metabolic activity amongst other functions.

A stem cell ‘niche’ is a highly specialized microenvironment that maintains the stem cells either indifferentiated or encourage specific cell differentiation towards one particular lineage/fate.

It is likely that different ‘stem cell niches’ within the respiratory tract from proximal to the distal axis will play specific roles during lung regeneration and it is highly unlikely that one ‘niche’ will serve all purposes.\textsuperscript{1,2}

The vasculature is lined with flat cells called endothelial cells. In between the vasculature and the airways resides the ‘skeleton’ of the lungs, it is called ‘the interstitium’ and it is the supporting scaffold.
Multipotent stem cells found outside the lung that can repair the injured lung

Besides local tissue-specific stem cells with reparative properties, there is a cohort of stem cells found outside the lung. These cells may be recruited to enhance lung regeneration. The most important source of these cells is the bone marrow. The bone marrow harbours two different types of stem cells: (i) stem cells that can generate the blood/immune cell system and (ii) a different type of stem cell, located in the same bone marrow niche, but with different properties, that can leave the niche, circulate in the blood stream and lodge on injured sites like the lung. The mechanisms by which these extra-pulmonary stem cells exert their reparative role are still being investigated but the general consensus so far is that these stem cells release growth factors that act in a reparative capacity.\(^3,4\)

Pluripotent stem cells for lung repair

Stem cells isolated from very early embryos can be used in regenerative medicine. These cells are able to produce all cell types seen in adults (hence, are called pluripotent) and have shown appropriate functional properties.\(^5,6\) Stem cell fate to a particular lineage, e.g. epithelial cells lining the respiratory airways, are heavily influenced, both \textit{in vivo} during development and \textit{in vitro} by a cocktail of regulatory molecules and cell/cell interactions. Hence, the way the cells are grown in the laboratory is important. Conventional two-dimensional cultures provide a lesser physiological environment. Growth of tissues in a three-dimensional (3D) setting, simulating what is happening during normal development, is important. Using stem cells grown three dimensionally, our group has been able to obtain specific lung cells from these pluripotent cells and demonstrated their functional properties in the test tube.\(^7\) Thus, having large quantities of these pluripotent cells in laboratory dishes (cell lines) can be a useful tool for toxicology testing. Products in the form of sprays (hairsprays and others) can produce inhalation problems. The EU in the near future will outlaw the testing of these products in animals, so \textit{in vitro} cell lines will be used as the expected norm of testing. Recently, a similar type of pluripotent stem cell has been obtained without the need to use early embryos. These cells are called induced pluripotent cells (iPS cells)\(^8\) and are likely to provide an alternative source to embryonic stem cells the use of embryos. The methodology is being improved at a rapid pace to ensure that no carcinogenic properties are present in these cells.
Lung repair

Animal models of lung disease

Several groups have developed appropriate animal models and tested the ability of stem cells to repair the injured side. A variety of stem cells have been used for that purpose including ‘adult’ stem cells obtained from the bone marrow, umbilical cord, circulation and found that administration of the cells, either as lung precursors or fully differentiated lung cells ameliorated the symptoms in animal models.\textsuperscript{9,10}

Regeneration by engineering whole lung preparations

Engineering lungs \textit{in vitro} is a mammoth task, due to the complexity of the lung structure, the variety of different cell types present in the respiratory tract, the flexible mechanics of the lung and the intricate function of respiration: removal of carbon dioxide from the circulation and allowance of oxygenation. Tissue engineering\textsuperscript{11} is the creation of living, functioning three-dimensional tissues or organs, utilizing specific combinations of cells, scaffolds and other signals. The scaffolds for lung tissue need to be flexible, stretchable and compressible during breathing. Finally, after formation of new lung tissue, the scaffold should degrade and the degradation products should not be toxic or pro-inflammatory. In brief: the task involves the use of cells, principally stem cells and especially designed materials, being naturally present, like collagens, or others man-made materials, like polymers.\textsuperscript{12} Three-dimensional scaffolds play an important role in tissue engineering to control cell function and to promote the formation of new tissues or organs.\textsuperscript{13} The scaffolds provide initial support to the seeded cells, localize the cells in the appropriate spaces, provide physical and biological cues for adhesion, migration, proliferation and differentiation, and assemble the propagated cells and secreted matrices into functional tissues and organs. Ultimately, the scaffold will be replaced by the cells’ own matrices during regeneration. Therefore, the scaffolds should be compatible, biodegradable and provide appropriate signals for the seeded cells to orchestrate the process. This complex is then grown in especially designed chambers, termed bioreactors\textsuperscript{14–16} where lung physiological conditions are best created and maintained, including correct levels of oxygen and other nutrients and appropriate physical and mechanical forces. As of today no fully \textit{ex vivo} fully grown and functioning lungs have been created and ready for implantation. However, specific and fully functioning segments of the respiratory tract have been engineered, e.g. the upper of the lung: the trachea.\textsuperscript{17}
A number of publications have recently appeared that demonstrate the usefulness of a somewhat different approach.\textsuperscript{18, 19} Researchers are now able to denude a lung of the cells lining the airways. These cells, if implanted into the host will be considered ‘foreign’ to the host and will be rejected. Researchers have successfully denuded the lungs and use the interstitial tissue as a frame or scaffold to allow host’s cells to repopulate this frame. The newly repopulated engineered lung will be easily recognized by the host, since the cells are of ‘host’ origin. Once the ‘lung scaffold’ is denuded of the potentially immunogenic cells, it is placed in a specially designed ‘bioreactor’ whereby epithelial cells which normally line the airways and endothelial cells which normally line the blood vessels are encouraged to repopulate the denuded ‘scaffold’. Interestingly, the seeded epithelium displays a remarkable hierarchical organization within the ‘scaffold’ and the endothelial cells again remarkably repopulate the denuded blood vessels. Researchers have been able to implant this newly engineered lung into experimental animals and to demonstrate that functionality is successfully achieved. This later approach takes the field of lung tissue engineering a step forward. The decellularized lung keeps the similarity of composition, biochemical properties, microstructure and shape of the native lung.

In addition researchers have been able to make use of discarded explanted lungs.\textsuperscript{20} When a donor lung becomes available, many times it is not suitable for transplantation, due to the marked inflammation observed. It is now possible to bathe the lungs outside the body in a normal physiological solution at body temperature and then inject, through the large airway, an attenuated adenovirus (or ‘cold’ virus) with the anti-inflammatory agent. These lungs loose their inflammation, the injured cells begin to repair and the lungs are ready for implantation.

**Challenges**

Many challenges lie ahead:

1. Number of cells needed for cell therapy: this challenge is frequently underestimated, the sheer number of cells needed, when there is tissue loss, is staggering and equivalent to 100 standard 10-cm tissue culture dishes. The scale-up procedure should progress from cells in a culture dish to a three-dimensional multi-cellular structure. Here, another challenge becomes apparent, it is important to overcome and limit the distance for oxygen diffusion.
(ii) Mode of cell delivery: this is also an area that needs further research. Would the cells be delivered in the circulation in the hope that they will home into the injured lung? Or would it be best to deliver them through the windpipes?

(iii) Does the cell home to the injured tissue and perform the required function? Again this is an area of intense research and it is not yet clear whether the delivered cells will become lung cells when they home into the appropriate tissue or will they deliver 'growth' factors to stimulate regeneration?

(iv) Cell survival: would the cells survive after homing? Or would the diseased micro-environment be adverse to cell survival? Again more research is needed.

(v) Translational research: a significant emphasis must now be placed on developing and standardizing techniques aimed at making the process of stem cell engraftment safe and efficacious, in addition to using rigorous in vivo pre-clinical testing in animal models.

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References


