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Stemness specificity of epithelial cells – application of cell and tissue technology in regenerative medicine

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Abstract

Stem cells are cells that have the potential to replicate and/or differentiate, becoming any tissue. This process could be theoretically repeated indefinitely and can be used to create or fix damaged parts any organ. There are many *in vivo* factors that cause stem cells to replicate and differentiate. Many of these interactions and mechanisms are still unknown. *In vitro* models have been successful in inducing stem cells to differentiate into the desired lineage using controlled methods. Recently, epithelial tissue has been successfully created using scaffolds on which stem cells are grown *in vitro* and then transplanted into the host. This transition creates significant problems. This is because *in vitro*-grown stem cells or stem cell-derived tissues are created in an isolated environment where virtually every aspect can be monitored and controlled. *In vivo* monitoring and controlling is significantly more difficult for a plethora of reasons. Cells in the body are constantly exposed to many signals and molecules which affect them. Many of the mechanisms behind these interactions and reactions are known but many others are not. As the corpus of knowledge grows, stem cells become closer to being applied in a clinical setting. In this paper, we review the current evidence on stem cell therapy in regenerative medicine and some of the challenges this field faces.

Running title: Stemness specificity of epithelial cells

Keywords: stem cells, epithelial cells, regenerative medicine

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Introduction

Organ systems in humans may become irreparably damaged due to ineffective regenerative capabilities and/or irreparable cellular, genetic damage or build up of harmful substances. Tissue transplantation has been a prominent mean of replacing damaged organs or tissue. Even though it has yielded great success, it also presents some unique problems. Graft rejection is a significant problem in the case of xenografts and allografts. Autografts do not always allow replacement of more complicated organ systems like kidneys or lungs. Furthermore, transplanting more complex tissue (e.g. brain) in human models is difficult as there are many interactions that could occur with the foreign and the native tissue. Engineering synthetic tissues and gene therapy are two alternative/complementary approaches to tissue transplantation. However, even though many advances have been made thus far both subjects are still in their infancy. Stem cells seem to display the greatest potential. This is because they could potentially differentiate into any tissue. Depending on where they were sourced, stem cells could also be autologous eliminating any potential risks for rejection. Thus far stem cells have been successfully used in some experimental models [1–3]. However, the widespread clinical application is still not suitable due to various factors such as ethics, legality and lack of knowledge. This is due to the complex nature of stem cells and the various factors that could cause their potentiation, differentiation and specialization.

Stemness plasticity and specificity of *in vitro* cultured cells

The progress in embryonic stem cell culture and the discovery that stem cells isolated from an adult body can differentiate into different cell types have led to an interest in their plasticity and its use in transplantation. Various sources of stem cells are used: human embryos, umbilical cord blood, bone marrow, skin epithelia, neural tissue and blood of the mature organism [4–7]. Stem cells must be kept in undifferentiated culture in order to utilize their full potential. Therefore, research is being carried out on growth factors and plasma proteins that maintain the phenotype of non-differentiated cells. All stem cells have properties for unlimited divisions throughout ontogenesis, as well as the ability to differentiate. Stem cells are multipotent because they can specialize towards more than one type of daughter cells. Adult stem cells are also present in an undifferentiated state in tissues. They can give rise to specialized and differentiated tissue based on their location [6, 7].

There are two methods for obtaining differentiated cells from stem cells. The first method requires the asymmetrical division of a stem cell into a stem cell and a cell which will differentiate. Stem-cell lines are grown and maintained at a specific temperature and atmospheric conditions (37 ^{o}C and 5% CO_2) in incubators. Culture conditions such as the cell growth medium and surface on which cells are grown vary widely depending on the specific stem cell line [8]. Different biochemical factors can be added to the medium to control the cell phenotype - for example, to keep stem cells in a pluripotent state or to differentiate them to a specific cell type [9]. Embryonic stem cells have the ability to differentiate into more cell types than adult stem cells. Differentiation is triggered by various factors *in vivo*, some of which can be replicated in *in vitro* stem cell cultures. The nature of stem cells necessitates the use of special culture media and reagents. Since suboptimal media may change the differentiation potential of stem cells, it is vital to choose the correct stem cell validated media and reagents at the start of the research process [8].

There are high hopes for using stem cells in cell therapeutic methods. Currently, stem cell lines are used in regenerative research and medicine. They can be used to study stem cell biology and early human development. In the field of regenerative medicine, it has been proposed to use stem cells in cellular therapies to replace damaged or diseased cells and tissues.

Epithelial tissue

Epithelial cells have recently been the subject of intensive research in many laboratories. These cells have proved to be a particularly appropriate model for investigations concerning the polarization of cell structure and functions of the cellular cytoskeleton, cell membrane and responses to many external factors. However, it is the very nature of epithelium itself that complicates this endeavour. Epithelia are tissues that limit and cover the surfaces of body cavities and external surfaces of animal organisms. These types of tissues are derived from all of the embryological germ layers: ectoderm (e.g., the epidermis); endoderm (e.g., the lining of the gastrointestinal tract) and mesoderm (e.g., the inner linings of body cavities). There are three principal shapes of the epithelial cell: squamous, columnar, and cuboidal. These can be arranged in a single layer of cells such as simple epithelium, squamous, columnar, cuboidal and pseudostratified columnar. Epithelial tissues can also be arranged in layers of two or more cells such as stratified (layered), which can be either squamous, columnar or cuboidal [10].

Epithelium lines outside and the inside body cavities. The outermost layer of skin is composed of dead stratified squamous and keratinized epithelial cells [11]. Tissues that line the inside of the mouth, the oesophagus, the vagina, and part of the rectum are composed of the nonkeratinized stratified squamous epithelium [12–14]. Other surfaces that separate body cavities from the outside environment are lined by simple squamous, columnar, or pseudostratified epithelial cells. Other epithelial cells line the insides of the lungs, the gastrointestinal tract, the reproductive and urinary tracts, and make up the exocrine and endocrine glands. The outer surface of the cornea is covered with fast-growing, easily regenerated epithelial cells. A specialized form of epithelium, endothelium, forms the inner lining of the heart, blood vessels and lymphatic vessels. Endothelium in the heart and blood vessels is referred to as vascular endothelium and in lymphatic vessels as the lymphatic endothelium. Another epithelial type, mesothelium, forms the walls of the pericardium, pleurae, and peritoneum.

The main functions of epithelial cells are related to their activity as covers of deeper layers of other cells and their effects in secreting and transporting various substances across the epithelium. Epithelium may be protective, absorptive, or secretory. It may produce special outgrowths (hairs, nails, horns on animals) and manufacture chemical materials (e.g., keratin), in which case the whole cell becomes modified. In other instances, it contains fat droplets, granules of various kinds, protein, mucin, watery granules, or glycogen. In a typical absorbing cell, granules of material are absorbed.

Renewal of epithelia takes place as a result of the proliferative activity of multipotent stem cells and/or unipotent progenitor cells. The lost cells are replenished as a result division of neighbouring cells. Stem cell proliferation and plasticity are controlled by epithelial-mesenchymal interactions and common signalling pathways [15]. The multilamellar epithelium is under continuous cell renewal as a result of mitotic divisions at the surface of the basal epithelium. In the body, epithelial cells do not show active migration, except for situations such as early embryonic development and organogenesis, cancer development or wound healing.

Application of differentiation potency of epithelial cells in advanced regenerative and reconstructive medicine

Regenerative medicine is an extensive branch of medicine that includes other fields such as bio-engineering, molecular biology etc. It is a field that is concerned with regenerating, repairing/replacing or creating lost or damaged tissue (be it cell or organ). The aim is to restore tissue function to normal. This aim could be achieved by stimulating the body's own repair mechanisms and/or creating organs and tissues *in vitro* and implanting them if the body is unable to [16]. However, there are many unknown mechanisms that act on stem cells within an organ or system. This makes controlled proliferation and differentiation of a desired lineage of cells difficult.

Recently, researchers have started investigating the use of stem cells to treat various pathologies of the respiratory tract. Chronic Obstructive Pulmonary Disease, abbreviated COPD, is an example. COPD is an irreversible inflammatory disease of the lungs. It occurs due to progressive damage of the alveolar walls, lung parenchyma and thickening of the small airways which traps air and increases airways resistance [17]. Two underlying pathologies are found in COPD. The first is emphysema and the second is chronic bronchitis. Emphysema occurs due to the destruction of the alveolar walls which results in a loss of elastic recoil. This process causes hyperinflation and enlargement of the airspaces distal to the terminal bronchioles. The second pathology, chronic bronchitis, occurs when there's a chronic and persistent mucus-producing cough for a period greater than three months during a minimum of two consecutive years. COPD can be fatal and impairs the lifestyle of many patients.

Under normal conditions, lung epithelium steadily repairs itself. Repair is carried out primarily by resident lung progenitor cells (different from embryonic cells that form lung tissue during embryonic development) [18]. However, circulating bone marrow-derived stem/progenitor cells may also contribute [19]. In a 2004 study, it was found that mice infected with H1N1 regain normal lung histology despite losing 40-50% of lung parenchyma due to severe inflammatory response [20]. Another study in 2011 demonstrated that when resident stem cells were replaced by isolated lung mesenchymal stem cells there was a decrease in the bleomycin-associated pathological development of pulmonary arterial hypertension [21]. However, repairing lung tissue is not as simple as introducing stem cells to a damaged area. One of the problems that researchers face is that there are many different kinds of resident stem cells which are found in various locations and niches throughout the same organ (e.g. submucosal glands and neurological epithelial bodies) [22]. Some of these cells express different genes which code for different products. Club cells (also known as Clara cells) produce Scgb1a1 while basal cells express p63+ transcription factor and cytokeratin 5/14 [23]. As a result, some of these differentiated cells behave differently when exposed to certain signals (e.g. hormones such as estrogen) and factors in their surrounding environment.

COPD is also complex due to the numerous variables that contribute to the pathogenesis of the disease. For example, many COPD patients are smokers. Smoking has been demonstrated to depress hematopoietic stem cell function [24]. This is relevant as the role of bone-marrow-derived stem cells in COPD repair is not known. Furthermore, COPD patients display elevated levels of myofibroblast proliferation in some compartments of the lung. This is further complicated by smoking since cigarette smoke promotes increased levels of miR-210 [25]. Increased levels of miR-210 are responsible for myofibroblast proliferation. Furthermore, some of the processes which are responsible for the elimination myofibroblasts (such as autophagy via targeting of ATG7) are significantly attenuated in COPD [22]. Introducing stem cells may cause unintentional myofibroblast differentiation and proliferation. This would increase fibrosis which causes remodelling instead of wound healing [25] and could possibly lead to tumour formation.

An area where stem cell therapy has been used clinically is to treat various tracheal defects. In such cases, stem cell therapy has been used on a compassionate basis as a viable option. Even though embryonic and adult stem cells can be induced to differentiate into the lung and respiratory epithelial cells *in vitro*, engraftment is rare after systemic administration [26]. According to Omori et al. using a three-dimensional bioengineered scaffold is vastly more successful in generating functional tissue [27, 28]. Bioengineered scaffolds can be used to regenerate skin, blood vessels, bone and cartilage. However, this technology is not perfect and there are still unforeseen problems in a clinical setting. Post-mortem studies from a female patient that received stem cell-seeded, decellularized tissue-engineered tracheal graft serve as a great example [27]. The patient died 3 weeks after the implantation due to intrathoracic haemorrhage which obstructed her airways. This case demonstrated to researchers that additional procedures, such as placing stents, had to be used as well. More importantly, it showed that some of the clinical complexities cannot be replicated in pre-clinical *in vivo* models and thus require greater consideration and further research.

Possible application and challenges of epithelial cells in human tissue

Thus far the sources of stem cells that have been examined are adult progenitor cells, embryonic stem cells, induced pluripotent stem cells and bone marrow-derived cells (particularly mesenchymal cells/ MSCs). Bone marrow stem cells are usually obtained by aspiration of bone marrow. Bone marrow aspiration provides a wide range of stem cell lineages. However, MSCs are most commonly studied [29]. MSCs are favoured as research material because they posses plastic-adherence and are capable of self-replication. MSCs can also be induced to proliferate and differentiate into products of the three germ layers [30]. Furthermore, these cells are capable of migrating to injured tissues (such as the lung), engraft and differentiate into one or various cells [31]. They are even capable of accumulating in damaged organs promoting regeneration [32, 33]. Another benefit of stem cells obtained from a patient's own bone marrow is that such cells would be autologous. This would eliminate the risk of rejection. Lastly, MSCs have been demonstrated to have antibacterial action, promote wound healing and protect against fibrosis and autoimmune disorders. MSCs inhibit bacterial growth by secretion of antimicrobial peptide LL-37 [34]. This initiates a cascade of reactions which ultimately activates macrophages. Wound healing is achieved by increasing IL-10 [34]. IL-10 down-regulates myeloperoxidase (MPO) production, attenuating neutrophil-mediated tissue damage. In a carbon tetrachloride-induced liver injury, MSCs were shown to aid in tissue repair by restoring Hmox-1, glutathione-S-transferase (GST), and nuclear factor-erythroid 2 p45 subunit related factor 20 (Nfr2) [35]. Thus, MSCs could be used in to repair damaged epithelium both directly by applying stem cells (as well as other necessary factors) to a damaged area or by minimizing harmful factors which damage epithelium. A problem with MSCs in a clinical setting is that proliferation and differentiation are highly dependent on various factors such as dosage, timing and route of administration [36]. MSCs may also be subject to various biochemical signals and pressures which are unknown in a clinical *in vivo* human model.

There is another type of stem cell that shows great potential. Adult stem cells are cells which are found within an adult animal body and are an essential part of the repair mechanism. Wada et al. [37] demonstrated that transplantation of alveolar type II cells into "pneumonectomized" mice engrafted and stimulated lung regeneration in the remnant lung. Until recently, adult progenitor cells were difficult to obtain in great quantities. However, scientists discovered a means of harvesting adult stem cells from adipose tissue. These cells, called adipose-derived stem cells (ADSCs), are very promising since they can be repeatedly harvested in mass quantities, require minimally invasive procedures and display low morbidity [29]. Ease of acquisition is an important clinical factor that both doctors and researchers must consider when comparing ADSCs to MSCs. Both ADSCs and MSCs share many similarities overall. They can both differentiate into the cells of all three germinal layers [38]. Furthermore, both show CD90, CD105, CD73, CD44 and CD166 surface markers and lack expression of CD45 and CD34 hematopoietic markers [29]. However, when ADSCs are compared to MSCs they display lower senescence and have higher proliferative capacity. Another positive aspect of ADSCs is that they are genetically and morphologically stable. Research data suggest that MSCs have a slight advantage where osteogenic capacity concerned [39]. On the other hand, ADSCs seem to be more efficient for collagen production [40]. ADSCs have been used by Akita et al. [41] to treat an intractable wound in the sacrococcygeal region resulting from radiation therapy. Akita's team healed the wound by using a combination of artificial skin substitute, human recombinant basic fibroblast growth factor and injected autologous ADSCs. In 2007, another study demonstrated that wound healing occurred in patients with severe and irreversible damage from radiation when they treated with ADSCs [42].

Embryonic stem cells (ESCs) are another group of stem cells that are heavily researched, similar to MSCs and ADSCs. Embryonic stem cells are unique as they have the capability to differentiate into any cell. An advantage of ESCs is that they can be continuously cultured without losing differentiation capability/pluripotency. Until recently many scientists thought that an embryonic cell's differentiation depended solely on external factors. Wolf et al. [43] used a fluorescent marker to detect levels of OCT4 in single cells over multiple generations. They wanted to determine how stem cells behaved when exposed to differentiation factors. Their results demonstrated that a cell's response to differential stimuli is dependent on levels of OCT4 and the time that it is "born". Maternal OCT4 levels play a significant role in determining how a cell will react to differentiation-inducing stimuli. A cell's reaction to an environmental cue could also be influenced by progenitor cells which are two to three generations/cycles old. However, *in vitro* animal models have also demonstrated that embryonic stem cells can also act in predictable ways. Wang et al. [44] demonstrated *in vitro* that embryonic stem cells could differentiate into corneal epithelial tissue. ESCs would differentiate into corneal epithelium if they were placed on the surface of deepithelialized superficial corneoscleral slices under the controlled conditions. Rippon et al. [45] obtained similar findings when trying to grow lung epithelium. Researchers have not been as successful with *in vivo* models. Once ESCs are committed to a cell lineage they are subject to a Hayflick limit, display phenotype abnormalities and a loss of differentiated characteristics [46]. More effective *in vivo* research is also limited by regulatory laws in different countries [47]. However, current *in vitro* models of ESCs could be helpful to test drugs, physiological stresses (e.g. hypoxia) and advance our understanding of genetic defects and stem cell interactions.

Ethical approval

The conducted research is not related to either human or animal use.

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Conflict of interest statement

The authors declare they have no conflict of interest.

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