In search of a non controversial source of stem cells for the reconstruction of corneal epithelium

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Abstract

Embryonic stem cells are unspecialised cells capable of dividing and renewing into specialised cell types. There are a lot of ethical and legal limitations to embryonic stem cell research. The main challenge to the embryonic stem cell scientists is to find ways to create stem cells without using vast quantity of human eggs, which are scarce. In regenerative medicine, scientists can harness the pluripotent ability of stem cells to replace damaged or deficient tissues. Stem cells exist in many adult tissues as well. Compared to embryonic stem cells, tissue specific stem cells have less self-renewal ability. Tissue specific cell lineages can develop into other cell lineages other than tissue of origin. Recent studies suggest that amnion derived from term placenta after birth may be a useful and non-controversial source of stem cells for cell transplantation and regenerative medicine.

In this study, I have reviewed the literature and suggest the possibility of bioengineering of corneal epithelial cells providing recovery of vision after corneal blindness. If successful this cell source will help in dealing with the immunorejection induced by allotransplantation.

Introduction

The stem cells are undifferentiated cells that are capable of self-renewal and differentiates into one or more cell lines. Embryonic stem cells are pluripotent cells forming all tissue types of organism (Pera et al 2000) and are isolated from the inner cell mass of the preimplantation embryos. These embryos are created in vitro using donated eggs fertilized in a lab. Most adult tissues in higher vertebrates contain stem cells that are responsible for maintaining homeostasis and (with limitations) cell turnover under normal and disease conditions. Adult stem cells do not have the range of differentiation properties that embryonic stem cells have, usually uni- or multipotential, forming the cell types of their origin. Adult stem cells like bone marrow cells or mesenchymal stem cells exhibit plasticity for colonizing a variety of tissues under experimental conditions (Herzog et al 2003). Scintu et al (2006) demonstrated that bone marrow stem cells can give rise to cells of different tissues including neural cells, hepatocytes and myocytes expanding their differentiation potential. Compared with embryonic stem cells, tissue specific stem cells have less self-renewal ability. Stem cells are identified by stem cell markers, which are specialized proteins, called receptors that will bind or adhere to other signalling molecules. The fate of the stem cells is controlled by intrinsic and extrinsic factors of which the latter are provided by the surrounding microenvironment, the so-called stem cell niche (Watt and Hogan). The microenvironment will influence the direction and the rate of the differentiation of embryonic stem cells, which is to be considered in the construction, differentiation and transplantation of systems for stem cells. Embryonic stem cells can be induced to form the lineage of interest by a combination of growth factors and/or their antagonists (Loebel, et al 2003). Figure I shows how stem cells can be differentiated.
Figure 1 shows how stem cells can be differentiated (http://stemcells.nih.gov/info/basics/basics3.asp)

Stem cell properties appear to decrease in a graduated fashion as cell lineage matures rather than losing them abruptly at a certain point during the process of differentiation (Blau et al 2001).

Xue et al (2005) reported the prospect of human embryonic stem cells in the clinical treatment of cardiac injury. Islet like clusters from spontaneously differentiating human embryonic stem cells were produced by Segev et al (2004). It is now possible to direct the differentiation of embryonic stem cells into lineages of therapeutic interest and to enrich cultures for cell types of interest in transplantation and drug screening. Whatever be the source, whether embryonic, fetal or adult origin, the donor sources should be screened for infections diseases, and suitability for use in the context of a particular clinical situation. There are many technical hurdles in stem cell research. Adult stem cells are quite hard to find, to extract sufficient cells for culture. They may be difficult to grow into large batches of undifferentiated cells in the laboratory. The main technical challenge facing embryonic stem cell research is that, even though it is easier to grow them into large batches, the stimulus that trigger their differentiation is not fully understood. Also, there is the possibility of immune rejection after transplantation into the recipient. There is always a chance that the transplanted cells could overgrow and cause cancer. In addition to the technical problems, there are ethical issues as well. The use of embryos for research has caused outrage from pro-life groups. They believe that destroying an embryo is immoral and unethical. Ethical issues are more when cloning is involved, where the embryonic stem cells are obtained by therapeutic cloning. In this technique, the nucleus of the egg cell is replaced with the nucleus from another cell in the body to develop an
embryo, from which the stem cells with specific properties are harvested, such as new gene. So supporters of embryonic stem cells started looking for less controversial alternatives to stem cells and had found a potential source in placentas saved during childbirth. Pluripotent stem cells were identified in cord blood (Kogler et al 2004) and multipotent mesenchymal stem cells were detected in various placental tissues (Takahashi et al 2004). Miki et al (2005) reported that the amnion contains cells with significant plasticity and differentiation potential. In this paper I am reviewing the literature and analysing the potential of amniotic epithelial cells to differentiate into corneal epithelium for transplantation to treat corneal disorders.

This paper suggests the possibility of developing a new strategy for generating corneal epithelial cells from amniotic epithelial cells in vitro, which can be used for the successful reconstruction of damaged corneas by transplantation of the amnion derived epithelial progenitor cells.

**Discussion**

Cornea is the outermost part of the eye and serves as a barrier and provides an optical function, transmitting and focusing incident light on the retina. The corneal epithelium is a nonkeratinized epithelial multiplayer that covers the anterior surface of cornea. Corneal epithelial cells are maintained by the centripetal migration of corneal epithelial stem cells located in the basal epithelium of corneoscleral limbus called limbus cells. Severe and widespread damage of the cornea in ocular surface diseases and injuries lead to loss of corneal and limbal epithelial cells. The therapeutic strategies for these conditions involve the transplantation of limbal grafts taken from the healthy contralaeral eye called limbal allograft (Morgan and Murray 1996), transplantation of cultured epithelial cells invitro (Koizumi et al 2002) and autologous transplantation of oral mucosal epithelial cells on amniotic membrane (Nakamura et al 2003). Whether used via allotransplantation or autotransplantation, the availability of limbal stem cells is always the limiting factor for treatment of corneal disorders by cell transplantation (Yanling Ma et al 2006). Additionally, numerous individuals reject allogenic corneal tissue, and the supply of donated corneas may soon be reduced by the increasing number of refractive surgeries. These problems can be solved by the development of artificial and bio engineered corneas. Jiang et al (2002) showed that tissue specific stem cells can differentiate into a lineage other than the tissue of origin. Optimal culture conditions for the induction of differentiation of epithelial progenitor cells from embryonic stem cells for corneal transplantation was suggested by Homma et al (2004). Recently it has been reported that mesenchymal stem cells could be used as a new source for autotransplantation in the treatment of corneal disorders (Yanling Ma et al 2006).

Amnion derived from term placenta can be used as a non-controversial source of stem cells for cell transplantation and regenerative medicine (Miki et al 2005). Amnion forms one of the three layers of the placenta. Amniotic epithelial cells are formed from the epiblast and were shown to retain the pluripotent properties and differentiation potential (Miki et al 2005). Amniotic epithelial cells are shown to differentiate to mature neural cells (Kakishita et al 2000). Miki and his co workers (Miki et al 2005) showed that the amniotic epithelial cells express the pluripotent stem cell specific transcription factors/markers, Oct-4 and nanog. They tested these cells by incubating them in culture dishes with various compounds and got them to form
heart cells, nerve cells, liver cells and pancreatic cells. Like nerve cells, cornea is also formed from surface ectoderm during development. So it may be possible to generate corneal epithelial cells from the amniotic epithelial cells. For this, the amniotic epithelial cells should be removed from the amniotic membrane as described by Miki et al (2005) and cultured on a suitable culture dish with an optimum culture medium and suitable growth factors which will provide sufficient corneal grafts for transplantation. These cells may be maintained and controlled by intrinsic and extrinsic factors in their local microenvironment, the so-called stem cell niche. Therefore characterisation of the microenvironment has to be explored for the successful culture.

Amniotic epithelial cells do not require other cell derived feeder cells to maintain the Oct 4 expression (Miki et al 2005) which is definitely an advantage over embryonic stem cells which require animal materials for their derivation and/or maintenance that will increase the likelihood of graft rejection. The absence of telomerase in the amniotic epithelium will prevent it from growing indefinitely, however it can be an advantage because telomerase is associated with tumors. It has been shown that there is no evidence of tumorigenicity in humans when isolated amniotic cells were transplanted into human volunteers to examine their immunogenecity (Sakuragawa et al 1992).

The main aim of this paper is to suggest the possibility of using a noncontroversial biological source to generate bio engineered corneal epithelial cells, free from antigen presenting cells that may be tolerated and may be better than tissue allografts now in use.

**Conclusion**

Placental tissue routinely discarded as medical waste, could provide an abundant non-controversial source of amniotic epithelial cells for culturing corneal epithelium, which can be used to repair severe vision loss caused by damage of cornea. Optimising this potential will be a big step forward in developing lineages of therapeutic interests and to enrich cultures in vitro for clinical transplantation and drug screening. Using the amniotic epithelial cells for culturing corneal epithelial cells will help to overcome immune rejection. This new source of stem cells may circumvent possible complications and provide sufficient material for surgical reconstruction after ocular damage. If successful, this source of cells could be used to develop a wide variety of tissues that can be used in regenerative medicine.
Reference:

