CHAPTER 1

INTRODUCTION

Rationale of the study

Cornea is the transparent structure at the front part of the eyeball that is essential for good vision. The outermost of the cornea is covered by corneal epithelium, the nonkeratinized stratified squamous epithelium, that can be renewed by limbal stem cells which located at corneoscleral junction (limbus). The lack of limbal stem cells (LSCs) is known as Limbal Stem Cell Deficiency (LSCD) that can be occurred due to primary or secondary causes. LSCD can lead to conjunctivalization to the cornea, corneal vascularization, corneal scarring and persistent corneal epithelial defect (Kruse, 1994; Puangsricharern and Tseng, 1995). Treatment of LSCD can be accomplished by transplantation of healthy limbal epithelia by limbal autograft from the contralateral eye in patients with unilateral LSCD, or by limbal allograft from cadaveric or living related eye in patients with bilateral LSCD (Kenyon and Tseng, 1989; Tsubota et al., 1995; Dua, 1998; Rao et al., 1999; Dua and Azuara-Blanco, 1999, 2000b). However, these techniques have some limitations. Using autologous tissue, only a small size of limbal tissue can be obtained from the healthy eye in order to prevent injury to the unaffected eye (Holland and Schwartz, 2000). In the case of allogeneic tissue, long-term use of immunosuppressive drugs is required to increase graft survival (Holland et al., 2003). The success in treatment of LSCD has increased since cultivated limbal stem cell transplantation was introduced by several groups of researcher (Pellegrini et al., 1997; Tsai et al., 2000; Koizumi et al., 2001; Rama et al., 2001; Nishida, 2003). A small limbal biopsy was used for generated the cultivated limbal stem cell sheet. Human amniotic membrane (HAM) was used as a culture substrate. The cultivated sheet was then transplanted to ocular surface with LSCD. However, treatment of bilateral LSCD using allogeneic tissue grafts are still face the problem of tissue rejection, patients needed prolonged postoperative immunosuppressant therapy that reduces the patients' quality life (Liang et al., 2009). To avoid these problems, an alternative source of autologous epithelial tissue was considered. Recently, cultivated oral mucosal epithelial transplantation (COMET) has shown promising results in reconstructing the corneal surface of patients with LSCD (Ma et al., 2009; Liu et al., 2011).

During LSCD treatment, cultivation and transplantation failures may occur, or subsequent surgery may be needed. Thus, cryopreservation may be beneficial in preserving the tissues or cells for future use in order to re-culture new epithelial sheets. Other benefits include expenses reduction, prevention of suffering of the patients during the biopsy procedure. In cryopreservation processes, it is importance to concern about the selection of cryoprotective agents (CPAs), cryopreservation methods, cooling and thawing rates as well as cellular types for prevention of ice crystal formation, osmotic stress and toxic effect of CPAs that causing cell injury during freezing (Davis, 2002). The conventional method called slow-freezing method may cause ice crystal formation that causes cell damage (Frederik and Busing, 1981). On the other hand, the vitrification method shows greater benefits because there is no ice crystal formation. The vitrification process uses a combination of a high concentration of CPAs and high cooling rate to reduce ice crystal formation as well as osmotic and toxic effect (Vajta et al., 2009). Dimethyl sulfoxide (DMSO) and glycerol are the most widely used CPAs. Moreover, Rich and Armitage reported that 25% propylene glycol is a potential component of a vitrification solution for corneas (Rich and Armitage, 1990). Kartberg and colleagues also reported that the cell damage was lower when cryopreserved by DMSO containing vitrification solutions compared with DMSO-free vitrification solutions (Kartberg et al., 2008). The samples that were mostly used in cryopreservation were an expanded limbal stem cell sheet or isolated limbal stem cells preserved by conventional methods using 10% DMSO (Kito et al., 2005; Oh et al., 2007; Yeh et al., 2008; Qu et al., 2009). These techniques needed to cultivate the cells prior to preservation in order to multiply the number of stem cells. Bratanov and colleagues reported that cryopreserved limbal tissues using 10% DMSO were able to proliferate and migrate epithelial cells in vitro (Bratanov et al., 2009).

However, due to the advantages of vitrification method that were described above, the vitrification was also reported the better potential in post thawing survival rates than slow freezing for embryos cryopreservation (Kolibianakis et al., 2009). Therefore, this study aimed to generate the cultivated epithelial sheets from fresh limbal and oral mucosal biopsies for transplantation in rabbit model prior to use in human therapy. Moreover, cryopreservation by vitrification of limbal and oral mucosal tissues immediately after biopsy compare to cultivated cells of both tissues were studied which may enhance the benefits in future use. Cryoprotective solutions for vitrification were also investigated to find the most suitable formula. The morphology and the expression of stem cell and differentiated cell markers of both cultivated epithelial sheets and the transplanted ocular surface were also examined.

Purpose of the study

1. To cultivate rabbit limbal stem cells and oral mucosal epithelial cells on HAM.
2. To cryopreserve rabbit limbal and oral mucosal biopsies and study the tissue growth potential and cell characterization.
3. To cryopreserve rabbit limbal stem cells and oral mucosal epithelial cells and study cell viability, growth potential and cell characterization.

Education/application advantages

1. To provide the culture method in generating the epithelial sheet from rabbit limbal and oral mucosal biopsies before applying this technique for therapeutic use in human.
2. To provide the cryoprotectants and vitrification method for the storage of limbal and oral mucosal epithelial cells.
3. To provide the information for further studies regarding ocular surface stem cells cultivation.