Abstract

Limbal stem cell deficiency (LSCD) is a disease characterized by the deficiency of stem cells in the limbus, which are responsible for the homeostasis and renewal of the corneal epithelium. This disorder results in corneal neovascularization, chronical inflammation and opacification, which may lead to loss of vision. The most successful treatment is the transplantation of limbal tissue or cultured limbal epithelial cells (LECs) onto the damaged ocular surface. The human amniotic membrane (HAM) is used as the feeder of the LECs culture, as well as for the LSCD treatment. HAM is also widely used in clinical practice, particularly for the treatment of chronic wounds.

This dissertation is particularly concerned on cell therapy for LSCD, on preparation of cells suitable for grafting onto the ocular surface, on the improvement of the LECs culture conditions, and on the preparation of appropriate carrier for the transfer of cells onto the damaged cornea. During my work I have used a wide spectrum of methods, e.g. cell cultures (LECs, mesenchymal stem, amniotic epithelial, conjunctival epithelial, goblet and 3T3 cells), immunohisto- and immunocytochemistry, microscopy, proliferation and colony forming assays, reverse transcription and quantitative real-time PCRs and statistical analysis.

For the improvement of the safety of LECs culture we prepared a protocol with the use of xeno-free media. This method could be used in transplantations for LSCD therapy, as this treatment is still missing in the Czech Republic. Moreover, we prepared the culture of conjunctival epithelial and goblet cells from corneoscleral rims for the treatment of conjunctival defects. We also demonstrated enhanced differentiation of murine mesenchymal stem cells into corneal-like cells by using of insulin-like growth factor-I with the corneal extract, as a possible future treatment of bilateral LSCD.

We established a new laboratory decontamination solution for the HAM decontamination as a possible substitution of a commercially available reagent. This procedure is already in use for the preparation of HAM for ocular transplantations and for the treatment of nonhealing skin wounds. We also prepared undamaged de-epithelialized HAM and viable amniotic epithelial cells by removing of the epithelial layer from the HAM after trypsin/EDTA treatment. The denuded HAM can be used for cell culture and epithelial cells stay viable for other methods of tissue engineering.

Key words: cornea, limbal stem cells, conjunctiva, mesenchymal stem cells, differentiation, amniotic membrane