

## **Abstract**

Part I: Endothelial cells form the posterior layer of the cornea and are important for maintaining its transparency. Dysfunctional endothelium can only be restored by transplantation. The global shortage of donor corneas requires the search for alternative treatments. The preparation of the graft by tissue engineering methods is complicated by low proliferative capacity of endothelium. To date, no endothelium-specific marker has been defined and the existence of endothelial stem cells has not been confirmed yet.

We have prepared a protocol for culturing endothelial cells from research-grade tissue - corneoscleral rims obtained after transplantation or corneas excluded from the transplant process. We monitored localization of selected proteins, including stem cell markers, in native tissue and in primary cell cultures. We prepared up to 6.4 cm<sup>2</sup> of endothelium from one cornea/rim, which had cellular features comparable to the native endothelium. This approach can increase the amount of endothelium for research or transplantation purposes. Using indirect immunohistochemistry, we showed that none of the previously proposed endothelial molecular markers is specific for these cells. We detected the expression of stem cell markers throughout the endothelial layer. In the porcine cornea model, we monitored its repair capacity after inducing central damage to the endothelium. We found that the repair is not affected by the presence of peripheral endothelium, but mainly by the length of organ culture and higher amounts of serum in the medium.

Part II: The human amniotic membrane (amnion) is a placental membrane that has a great potential in the treatment of non-healing wounds of various etiologies. The mechanism is acceleration of granulation and epithelialization of the defect. In the Czech Republic, amnion therapy has been standardized so far only for the reconstruction of the ocular surface. An important part of amnion preparation is effective but non-toxic decontamination. In clinical practice, intact amnion is mainly used for transplantation, but de-epithelialized amnion is used preclinically as a carrier for culturing cells for transplantation purposes.

We prepared our laboratory decontamination solution with properties (high microbiological efficiency, low toxicity) similar to a commercial solution and developed a protocol for amnion de-epithelialization, with which we obtained both amnion with an intact basement membrane, but also vital epithelial cells, which can be further used by tissue engineering methods. Based on preliminary results, we show a positive effect of amnion on healing of non-healing skin wounds in patients enrolled in a multicenter study.

**Key words:** cornea, endothelium, amniotic membrane, stem cells, cultivation, immunohistochemistry, transplantation