

Transplantation of *ex vivo* expanded stem cell amniotic sheet for ocular surface reconstruction

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To surveil the outcome of a new technique of *ex vivo* expanded stem cell allograft for limbal stem cell deficiency (LSCD). The limbal stem cells were separated from limbal tissue of male goats, the primary limbal stem cells identified by flow cytometric analysis were expanded, examined the stem cell relevant properties and cryopreserved in liquid nitrogen. The cryopreserved corneal limbal stem cells were thawed then transplanted onto the top of a cell-free human amniotic membrane framed on a nitrocellulose sheet and grown for 12-14 days in culture medium to construct corneal epithelium sheets. The corneal epithelium thus generated from limbal stem cells was transplanted into the right eye of pathological models of total LSCD that had been created in 15 female goats. Then the clinical observation and histological examination were used to evaluate the effects of the reconstruction of corneal epithelium. Polymerase chain reaction analysis was used for detection of the SRY gene to identify whether male goat limbal cells exist in the female goat for a long time. During the six-month recovery period, we found that the corneal epithelial status of the experimental goats was improved gradually. The SRY gene of the male goat could be found in the early two months after transplantation. The results showed that *ex vivo* expanded stem cell allograft is a useful technique for restoring the ocular surface in profound LSCD. The allograft play a key role to incorporate with progenitor cells to repair the damaged goat cornea.

Keywords: artificial corneal epithelium, amniotic membrane, limbal stem cells, allograft

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