

CORNEAL RECONSTRUCTION WITH TISSUE-ENGINEERED CELL SHEETS COMPOSED OF HUMAN IMMATURE DENTAL PULP STEM CELLS

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Introduction Limbal stem cell deficiency (LSCD) is caused by a variety of diseases, such as cicatricial keratoconjunctivitis (Stevens-Johnson syndrome, alkali burn, ocular cicatricial pemphigoid), genetic disorders (aniridia) and others. Limbal stem cells (LSC) are defined as self-renewing, highly proliferative cells *in vitro*, which express a set of specific markers and presented the capacity to reconstruct *in vivo* the entire corneal epithelium in case of ocular surface injury. Currently, LSC transplantation is a commonly used procedure, in patients with both uni- and bilateral LSCD. Although LSC transplantation holds great promise for patients, several problems need to be overcome. In order to provide a new source of autogenous and allogeneous cells for ocular surface reconstruction, we aimed to analyze whether human immature dental pulp stem cells (hIDPSC) could present similar key characteristics with LSC. Further we investigate the outcome of tissue-engineered cell sheet composed of hIDPSC in an animal model of LSCD after chemical burn injury.

Methods Human hIDPSC were cultured according to previously described protocol (Kerkis et al., 2006). In order to verify LSC markers (ABCG2, integrin β 1, vimentin, p63, connexin 43 and keratin 3/12) expression in undifferentiated hIDPSC immunohistochemistry and Reverse Transcription Polymerase Chain Reaction (RT-PCR) were used. The LSCD was induced by chemical burn with NaOH 0.5M applied for 20 seconds (mild chemical burn, MCB: n=8) or for 40 seconds (severe chemical burn, SCB: n=7) in one eye of male rabbits. Superficial keratectomy was performed in order to remove the fibrovascular pannus that covered the animal burned corneas. Tissue-engineered hIDPSC sheet was transplanted to the corneal bed and then covered with a patch of desepithelialized human amniotic membrane (AM) in both MCB (n=5) and SCB (n=4). In the respective control groups, the denuded corneas were covered with the AM patch in the same way but without the hIDPSC. The corneas were submitted to histological, transmission electron microscopy (TEM) analysis and immunohistochemical study using anti-human corneal tissue specific antibodies.

Results We showed that hIDPSC continuously expressed markers of LSC such as ABCG2, integrin β 1, vimentin, p63, connexin 43 and keratin 12, being negative to corneal cells marker keratin K3, when cultured *in vitro*. Three months after surgery, corneal transparency of the eyes that underwent hIDPSC transplantation was improved throughout the follow-up while the control animals developed total conjunctivalization and opacification. The clinical data were confirmed by histological and TEM analysis that showed uniform corneal epithelium similar to the one of the normal corneas of the non injured eyes. The corneal tissue showed also positive immunostaining with anti-human antibodies against integrin β 1, cytokeratin-18, p63 and keratin-3. In the control corneas, as expected, none of these antigens were detected.

Conclusions Our data showed that hIDPSC share similar characteristics with LSC. Transplantation of tissue-engineered hIDPSC sheet was successful for the reconstruction of corneal epithelium in the animal model of LSCD. Our finding qualifies hIDPSC as an alternative source of the cells to LSC, which can be used for corneal reconstruction.