Abstract

Aims: Limbal stem cell (LSC) deficiency is one of the most challenging ocular surface diseases. The aim of this thesis was to study damaged ocular surface reconstruction. Therefore, a mouse model of limbal transplantation was established. Furthermore, LSC isolation, transfer of LSCs and bone marrow-derived mesenchymal stem cells (MSCs) on nanofiber scaffolds were studied.

Material and methods: Syngeneic, allogeneic and xenogeneic (rat) limbal grafts were transplanted orthotopically into BALB/c mice. Graft survival, immune response and the effect of monoclonal antibodies (mAb) (anti-CD4 and anti-CD8 cells) were analyzed. Mouse LSCs were separated by Percoll gradient; subsequently, they were analyzed for the presence of LSC and differentiation corneal epithelial cell markers and characteristics using real-time PCR and flow cytometry. Nanofiber scaffolds seeded with LSCs and MSCs were transferred onto the damaged ocular surface in mouse and rabbit models. Cell growth on scaffolds, post-operative inflammatory response and survival of transferred cell were analyzed.

Results: Limbal allografts were rejected promptly by the Th1-type of immune response (IL-2, IFN-γ) involving CD4$^+$ cells and nitric oxide produced by macrophages, contrary to the prevailing Th1 and Th2 immune responses (IL-4, IL-10) in xenografts. Anti-CD4 mAb significantly postponed the rejection in allografts and in xenografts. The lightest and densest fraction of the Percoll gradient were both enriched with cell populations with a high expression of SC markers and side-population phenotype. Contrary to the lightest (40%), the densest (80%) fraction contained K12$^-$/p63$^+$ cells with characteristics that were closer to SCs. In the mouse model, the nanofiber scaffolds with LSCs and MSCs suppressed the inflammatory reaction. In the rabbit model, the MSCs on nanofiber scaffolds reduced alkali-induced oxidative stress and significantly accelerated corneal healing.

Conclusions: Limbal grafts do not enjoy any privileged position of immunity in the eye. Anti-CD4 mAb treatment is a promising immunosuppressive approach after limbal allotransplantation. By centrifugation on Percoll gradient, two distinct populations of corneal epithelial cells with SC characteristics were separated, with the K12$^-$/p63$^+$ population being closer to LSCs. Nanofiber scaffolds can be useful for LSC and MSC transfer and future treatment of ocular surface injuries.

Key words: limbal stem cells, limbal stem cell deficiency, limbal transplantation, mesenchymal stem cells, nanofiber scaffolds.