

R1  R2  R3  PG0  PG1  Estagiário  Tecnólogo  PIBIC

Last Name - Melo

First Name - Gustavo

Middle - Barreto

Service (sector)

Cornea and External Disease

Nº CEP para Uso de Animais do Instituto Butantan, nº 250

Instituto Butantan

Immature Dental Pulp Stem Cells for corneal epithelium reconstruction. Melo, G. B.; Gomes, J. A. P.; Monteiro, B. G.; Maranduba, C. M. C.; Sant'Anna, O. A.; Cerruti, H.; Kerkis, A.; Kerkis, I. Departamento de Oftalmologia, Universidade Federal de São Paulo; Laboratório de Genética, Instituto Butantan.

Purpose: To compare the effect of undifferentiated immature dental pulp stem cells (IDPSC) isolated from deciduous teeth for ocular surface reconstruction in an animal model of total limbal stem cells deficiency (LSCD) by mild and severe chemical burns (MCB and SCB).

Methods: An animal model of LSCD was induced by chemical burn with NaOH 0.5M applied in one eye of New Zealand male rabbits either for 40 seconds (SCB) (n=4) or for 25 seconds (MCB) (n=3). After 1 month, a superficial keratectomy was performed. Human IDPSC, which had been previously cultivated, were transplanted to the corneal bed and then covered with a patch of amniotic membrane (AM). It was sutured with the epithelial side down to the episclera. In the control group, the denuded corneas were covered with the AM patch in the same way but without the IDPSC. After 2 months, a detailed clinical evaluation of the rabbit eyes was performed. The animals were then sacrificed, their eyes were enucleated and the corneas were submitted to histological analysis and immunohistochemical study with confocal microscopy. To assess the differentiation of the IDPSC, antibodies (AB) were used against keratin-3 (specific for human corneal epithelium) and IDPSC (made by our group, specific for human IDPSC). Cy3 anti-mouse AB was used as the secondary AB.

Results: Corneal transparency of the eyes that underwent IDPSC transplantation was improved throughout the follow-up. Rabbits from MCB group presented much clearer corneas with less neovessels than those from SCB and control groups. SCB animals disclosed slightly improved transparency in comparison to controls. The control animals corneas developed total conjunctivalization and opacification in both MCB (n=1) and SCB (n=2). The clinical data was confirmed by histological analysis that showed uniform corneal epithelium in MCB eyes; corneal epithelium partially covered by conjunctival epithelium in SCB eyes; and conjunctival epithelium over the corneal stroma in control eyes (both MCB and SCB). The presence of IDPSC was detected in both MCB and SCB animals. The differentiation for the corneal tissue was proven through positive staining of antibody against keratin-3. MCB and SCB rabbits showed the same pattern of staining. In the control animal corneas, as expected, none of these antigens were detected.

Conclusions: Our results suggest that IDPSC transplanted to the eyes of a

rabbit model of TLSCD have the ability to migrate, proliferate and differentiate into corneal epithelium after both mild and severe chemical burns. The former presented better clinical results than the latter. However, both showed the same results at confocal microscopy. The authors believe that even severely chemical burnt corneas may benefit from IDPSC transplantation.