( ) R1 ( ) R2 ( ) R3 (X) PG0 ( ) PG1 ( ) Estagiário ( ) Tecnólogo ( ) PIBIC Last Name - Monteiro First Name - Bábyla Middle - G.

Service (sector) Instituto Butantan - Comitê de Ética para Uso de Animais do Instituto Butantan, nº 250.

Differentiation of immature adult stem cells isolated from human dental pulp into corneal ephitelium in an animal model of total limbal stem cell deficiency Monteiro BG1, Maranduba CMC1, Melo GB2, Fonseca SAS3, Cerruti H4, Kerkis A3, Kerkis I1, Gomes JAP2. 1Laboratório de Genética do Instituto Butantan, 2Centro Avançado de Superfície Ocular (CASO), Instituto da Visão, Universidade Federal de São Paulo – Unifesp/EPM, 3Clínica de Reprodução Humana Roger Abdelmassih 4Clínica Cera

Purpose: To evaluate the role of dental pulp stem cells (DPSC) for ocular surface reconstruction in an animal model of total limbal stem cell deficiency (TLSCD). Methods: An animal model of TLSCD was induced by chemical burn with NaOH 0.5M applied for 40 seconds in one eye of male rabbits. Thirty days afterwards, a 360° peritomy followed by a superficial keratectomy was performed in order to remove the fibrovascular pannus that covered the animal burned corneas. In group A (n=4), human DPSC, which had been cultivated in accordance to a protocol described by Kerkis et al, were transplanted to the corneal bed and then covered with a patch of amniotic membrane (AM) which was sutured with the epithelial side down to the episclera using 10.0 nylon. In group B (control, n=1), the denuded corneas were covered with the AM patch in the same way as it was performed in group A, but without the DPSC. After 3 months, a detailed clinical evaluation of the rabbit eyes was performed under the surgical microscope. 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 DPSC, it was used antibodies (AB) against: p63, integrin β1, cytokeratin-18 (specific for human epithelium); keratin-3 (specific for human corneal epithelium); and anti-DPSC AB (made by our group, specific for human DPSC). Cy3 anti-mouse AB was used as the secondary AB. Results: Clinically, the corneal transparency of the operated eyes in group A was improved throughout the follow-up. In group B, the operated corneas had total conjunctivalization and opacification. The clinical data was confirmed by histological analysis that showed uniform corneal epithelium similar to the one of the normal corneas of the non injured eyes. The presence of DPSC was detected using the antibody anti-DPSC. The differentiation for the corneal tissue was proven through positive staining of antibodies against integrin β1, cytokeratin-18, p63 and keratin-3. In group B, as expected, none of these antigens were detected.

Conclusion: Our results suggest that DPSC transplanted to the eyes of a rabbit model of TLSCD have the ability to migrate, proliferate and differentiate into corneal epithelium. They may play a role in the treatment of many ocular surface diseases that are associated with limbal stem cell deficiency