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PIBIC Last Name - Pires First Name - Renato Middle - Teixeira Ferreira

Service (sector) Cornea and External Disease Nº CEP

Ex Vivo Preservation and Expansion of Human Limbal Epithelial Stem Cells on Amniotic Membrane. R.T.F. Pires1,2,3, D. Meller1,4, S.C.G. Tseng1, A-L.H. Lima2. Department of Ophthalmology, Bascom Palmer Eye Institute, University of Miami School of Medicine, Miami, FL, USA1, Universidade Federal de São Paulo, Escola Paulista de Medicina, São Paulo, SP, Brasil2, Instituto de Olhos de Goiânia, Goiânia, GO, Brasil3, Department of Ophthalmology, University of Essen, Essen, Germany4. Purpose: Amniotic membrane transplantation is effective in restoring the corneal surface by expanding the remaining limbal epithelial stem cells in patients with partial limbal stem cell deficiency. We thus wondered if such an action is also maintained ex vivo by amniotic membrane. Methods: Explant cultures from human limbus, peripheral cornea, and central cornea were established on amniotic membrane, and their outgrowth rate was compared. For outgrowth of human limbal epithelial cells (HLEC), cell-cycle kinetics was measured by BrdU labeling for 1 or 7 days. The 7 day labeled epithelial sheets were chased for 14 days in the same primary culture or after being transplanted to 3T3 fibroblast-feeder layers or subcutaneously into athymic Balb/c mice. Epithelial morphology was studied by histology and transmission electron microscopy. Its phenotype was defined by immunostaining with monoclonal antibodies to keratins and mucins. Results: No outgrowth (0/22, 0%) was noted in central corneal explants, while outgrowth was noted in 2 out of 24 (8.3%) peripheral corneal explants and 77 out of 80 (96.2%) limbal explants ($p < 0.0001$). Twenty-four hour BrdU labeling showed a uniformly low (i.e., less than 5%) labeling index in 65% of the limbal explants but a high (i.e., less than 50%) labeling index in 35% of limbal explants and all peripheral corneal explants. A high labeling index was noted in 61.5% of the limbal explants with the remaining still retaining low labeling index after continuous BrdU labeling for 7 days. A large number of label-retaining basal cells with a high labeling index were still noted following 14 days of chase in the primary culture or following transplantation onto 3T3 fibroblast-feeder layers or athymic mice. HLEC cultured on AM were strongly positive for K14 keratin and MUC4 and slightly positive in suprabasal cells for K3 keratin but negative for K12 keratin, AMEM2, and MUC5AC. After subcutaneous implantation into athymic mice, the resultant epithelium became markedly stratified and the basal epithelial cells were strongly positive for K14 keratin, while the suprabasal epithelial cells were strongly positive for K3 keratin and MUC4 but negative for K12 keratin and MUC5A/C. Conclusions: These data support that amniotic membrane cultures preferentially preserves and expands limbal epithelial stem cells that retain their in vivo slow-cycling, label-retaining, and undifferentiated properties. This culture system may allow transplantation of limbal epithelial stem cells using a small amount of the donor limbal tissue.