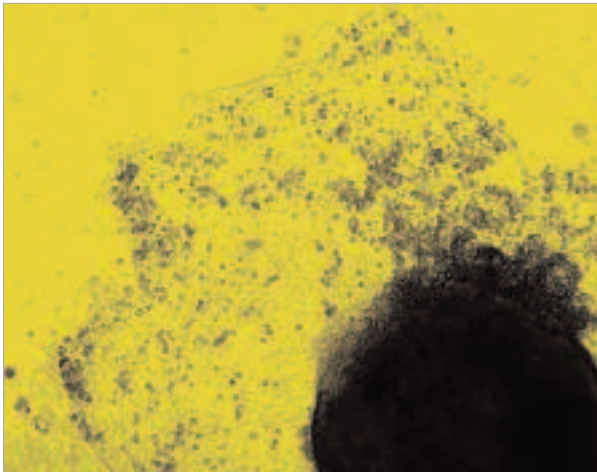


OCULAR SURFACE RECONSTRUCTION: BASIC, CLINICAL AND SURGICAL ASPECTS

Rubens Belfort MATTOS Júnior

Ophthalmology Department / Federal University of São Paulo (Unifesp)



Epithelial expansion from a limbal explant cultivated ex vivo (x40)

Our study includes the following objectives: (i) to evaluate the ultra-structural, biochemical and molecular aspects of the amniotic membrane colonized *ex vivo* by corneal epithelium; (ii) to analyze the alterations of the ocular surface by different specialized diagnostic methods; (iii) to evaluate new treatments for dry eye; (iv) to evaluate the efficiency of reconstructive surgery of the ocular surface by using amniotic membrane associated or not with limbal transplant in Ocular Surface Disorder. Briefly we present the methods used for each protocol: scanning transmission electron microscopy in fresh amniotic membrane preserved by two different methods; quantification by ELISA – in both preserved and fresh amniotic membrane – of growth factors (recombinant human epithelial growth factor (EGF), fibroblastic growth factor (FGF-4, FGF-basic), hepatocyte growth factor (HGF), keratinocyte growth factor (KGF), beta-transformant growth factor (TGF-b)), interleukins (IL-4 e IL-10) and prostaglandins (PGE2); identification and quantification of growth factors and cytokines mRNA's in the human amniotic membranes by PCR techniques, "Northern blot" and "Western blot"; use of the amniotic membrane in experimental cicatricial keratoconjunctivitis in rabbits; ultra-structural analysis of cell proliferation (immunofluorescence with Ki-67), apoptosis (morphological method with Hoechst 33342) and immunohistochemical/FACS to determine the expression of the epithelial.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

This project includes the development of 15 research projects [5 basic research (PE), and 10 clinical research (PCC)]. The resources provided by FAPESP allowed us to install the Advanced Ocular Surface Laboratory (CASO), the first cell biology lab in Brazil focused on the development of cell therapy technology for ocular surface reconstruction.

Obtained results to date: Ultra-structure characterization, biochemical and molecular aspects of the human amniotic membrane. The evaluation of the efficacy of amniotic membrane and limbal stem cell transplantation for ocular surface reconstruction. Graft survival was observed with a cumulative survival of 33% after a mean follow-up time of 33 months. Increase in postoperative visual acuity was observed in 60.6% of the operated eyes during this period. Marked impact on graft survival was observed for patients with Stevens-Johnson syndrome, dry eye, keratinization, eyelid abnormalities, and allogeneic conjunctival limbal transplantation (independently of HLA compatibility) ($p < .05$). Preoperative dry eye was the most important prognostic parameter for surgical outcome ($p < .001$).

We are also completing three important prospective, comparative studies evaluating the efficacy of amniotic membrane in the treatment of pterygia, bullous keratopathy and corneal and scleral thinning.

We developed at the CASO the technique for amniotic membrane colonization with human limbal epithelial stem cells expanded *ex vivo*. In our hands, the best method includes the use of fragments of limbal tissue (and not cell suspension) over EDTA deepithelialized human amniotic membrane. We are also evaluating different cell lines (conjunctival epithelia and immature dental pulp stem cells) in order to compare the survival and possibility for clinical application.

We started to transplant these *ex vivo* limbal epithelial cultivated amniotic membranes in patients with total limbal stem cell deficiency with reasonable results in the short-term. More cases are now scheduled to be operated in order to obtain a better evaluation of the procedure and survival of the transplanted cells.

We developed the technique of impression cytology as a diagnostic tool for limbal stem cell deficiency and other diseases. We are now standardizing immunohistochemical analysis with different cell and proliferation markers.

We are in the process of evaluating, through a double blind controlled study, the efficacy of topical use of 0.05% cyclosporine A in the treatment of dry eye secondary to Sjögren's syndrome.

Our thematic project is a multi-disciplinary project of the Federal University of Sao Paulo (UNIFESP) supported by FAPESP, with the participation of another two national and two international Universities (University of Nottingham, UK, and McGill University, Montreal, Canada).

MAIN PUBLICATIONS

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Rubens Belfort MATTOS Júnior

Universidade Federal de São Paulo (Unifesp)
Departamento de Oftalmologia
Rua Botucatu, 822 – Vila Clementino
04023-062 – São Paulo, SP – Brasil
+55-11-5085-2010
eyebr@webmail.epm.br