Regenerative medicine and cell therapy in ophthalmology - human amniotic membrane as a source for biocuratives and stromal/mesenchymal stem cells

Caroline Nascimento Barquilha[1,2], Matheus Schwengber Gasparini[2], Mariane Aparecida Risso[1,2], Helga Caputo Nunes Holzhausen[1,2], Damiana Pedro De Oliveira[1,2], João Pedro Arantes Aun Migueis[1,2], Maria Laura Costa Do Nascimento[3], Ana Carolina Migliorini Figueira[1], Mônica Alves[2]

[1] Brazilian Biosciences National Laboratory (LNBio), Brazilian Center for Research in Energy and Materials (CNPEM), Campinas, SP, Brazil.
[2] Department of Ophthalmology, School of Medical Sciences (FCM), State University of Campinas (UNICAMP), Campinas, SP, Brazil.
[3] Department of Obstetrics and Gynecology, School of Medical Sciences (FCM), University of Campinas (UNICAMP), Campinas, SP, Brazil.

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Abstract: The most severe forms of ocular surface diseases (OSDs), like dry eye, chemical burns, and neurotrophic conditions, continue to pose significant treatment challenges[1]. In the context of regenerative medicine, the human amniotic membrane (AM) has proven to be an interesting biocurative for ophthalmological interventions, in addition to being a promising source of stromal/mesenchymal stem cells (MSC) for cell therapy[2]. AM is rich in growth factors, with cells presenting a high capacity for self-renewal and differentiation potential, besides the immunomodulatory and pro-angiogenic characteristics that favor tissue healing[3]. Thus, we aimed to systematize the production and thoroughly characterize two types of biocuratives derived from AM - cryopreserved (C-AM) and freeze-dried (FD-AM), as well as to isolate the MSC population (MSC-AM) with potential pre-clinical and clinical applications. AM samples were collected from five human placentas obtained from patients undergoing elective cesarean sections at the University of Campinas maternity hospital (CAISM-UNICAMP, Campinas, SP, Brazil – CAAE 45209521.4.0000.5404). Protocols for decontamination and processing under microbiological and quality control were standardized. The biocuratives (C-AM and FD-AM) were then characterized regarding morphology/ultrastructure (histological analysis and scanning electron microscopy) and functional properties (transparency, elasticity, transepithelial electrical resistance, biodegradation rates, and growth factor levels by ELISA assay). In addition, we have been characterizing the MSC-AM by their morphology, population doubling time (PDT), potential to tri-lineage differentiation, and immunophenotyping (cytometry). As results, microbiological control tests ensured the sterility of the final products. Compared to a fresh AM, the general ultrastructure and epithelial/stromal tissue layers of C-AM and FD-AM were preserved after processing. Furthermore, the biophysical and mechanical properties, such as transparency, elasticity, and transepithelial electrical resistance, were equally maintained in both biocuratives. The biodegradation test demonstrated that FD-AM was better preserved than C-AM after one month. Additionally, the profile of EGF, FGF2, HGF, PDGF-A/B, TGFβ1, and VEGF levels from protein extract and secretome of C-AM and FD-AM were different. Furthermore, the obtained MSC-AM population presented fibroblastoid morphology, typical of stromal/mesenchymal stem cells. Partial analyses showed that the average PDT from passages 3 to 8 was 38 hours. Preliminary results demonstrated

that MSC-AM were positive to CD90 (>90%) and negative to CD34/CD45 (<5%). Also, they were able to differentiate into osteogenic, adipogenic, and chondrogenic cells, highlighting the plasticity of MSC-AM. Together, our results reinforce the potential application of AM in regenerative medicine and cell therapy. This study contributes to the implementation of standardized processes to ensure the production of safe and highly qualified biocuratives and MSC, which can be used as advanced therapy products for treating OSDs.

References

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