Isolation method of human limbus-derived stromal/mesenchymal stem cells under quality control for pre-clinical and clinical ophthalmological applications

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Introduction

The severe forms of ocular surface diseases (OSD), as seen in cicatricial conditions, chemical burns, limbal cell deficiency, and neurotrophic disease, still impose therapeutic challenges and vision threats. In the precision medicine field, limbal stem cells (LSC) are a promising tool for cell therapy and ocular surface regeneration. Once there is no consensus about LSC isolation, we aimed to standardize a protocol of LSC isolation from donor scleral-corneal tissues for future pre-clinical and clinical applications.

Methods

Five scleral-corneal rings were collected after corneal transplantation in partnership with the Biobank of the Brazilian Biosciences National Laboratory (LNBio/CNPEM - CAAE 58792322.5.0000.5453) from Campinas, SP, Brazil. Several protocols of tissue decontamination were tested, followed by microbiological control analyses. We also tested two different enzymatic digestion protocols to isolate LSC, using Dispase or Collagenase I. For validation, cells were counted, and their morphology was evaluated.

Results

The standard protocol consisted of cutting the limbic area of the scleral-corneal rings into small fragments (3x3mm), washing with PBS, and incubating overnight with an antibiotic/antimycotic solution for decontamination, which was confirmed by microbiological analyses. For isolation of LSC cells, fragments were incubated overnight with Collagenase I in DMEM at 37ºC. The remaining tissue was dissociated, filtered, and centrifuged. The pellet of cells was resuspended, counted, and cultured. Cell counting demonstrated a viability of cell population over 98% since passage 0. The population of isolated LSC presented fibroblastoid

morphology, typical of mesenchymal stem cells. Three different researchers performed the protocol for validation, and the same results were obtained.

Conclusions

LSC can naturally regenerate the ocular surface and is a promising tool for composing new advanced therapy medicinal products to treat OSD. Considering that the sources for this cell type are limited, we highlight the importance of a proper isolation method. The implementation of the present protocol will allow an increase in the number of LSC available for pre-clinical and clinical applications.

References

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