

VALIDATION OF TWO TECHNIQUES TO OBTAIN LANGERHANS CELLS, SUITABLE FOR INTRODUCTION IN A IMMUNOCOMPETENT THREE-DIMENSIONAL HUMAN SKIN MODEL

Dayane Piffer Luco^{1*}, Bianca da Silva Sufi¹, Patrícia Santos Lopes², Maria Fátima Guarizo Klingbeil, Monica Beatriz Mathor¹

*dayaneluco@usp.br

¹IPEN - Instituto de Pesquisas Energéticas e Nucleares – Brazil; ²UNIFESP- Universidade Federal de São Paulo- Brazil,

An immunocompetent three-dimensional human skin model presenting *in vitro* more complex structures like melanocytes and dendritic cells represents a new generation for human skin substitute, allowing the studies of primary sensitization processes and skin sensitization, being able to overcome the need for animal models at least in the early stages of allergenicity tests of cosmetics and drugs. The setting of skin models are typically based on a de-epidermized dermis or collagen scaffold, supplanted with fibroblasts and keratinocytes.

This study presents the validation of two different techniques to isolate and cryopreserve of Langerhans cells (CL) to permit its subsequent introduction into the immunocompetent three-dimensional human skin model. After the epidermis mechanical separation and cell suspension by trypsinization, there were tested two different purification methods in parallel: (a) collecting the supernatant of the cultured keratinocytes before the first change of medium and (b) mechanical purification system based on cell separation according to density – Ficoll Paque Plus[®]. Both systems were evaluated for their efficiency in terms of purification and yield by phenotype using FITC conjugated with HLA-DR marker in flow cytometer. The first system was simple to apply, had high cell yield but low LC coefficient. The second system, although having higher complexity and requiring longer period to settle, brings higher degree of LC recovery. LC cryopreservation from both systems was performed using a rich fetal bovine serum cold-freezing medium. After thawing, the cell viability was checked and verified that the freezing method is suitable for this cell type.

Key words: Langerhans Cells, Ficoll, cryopreservation, immunocompetent three-dimensional human skin model.

1 - Resumo e apresentação de painel intitulado “Two techniques validation to obtain suitable Langerhans cells for an immunocompetent three-dimensional human skin model” no 1st Latin American Congress on Alternatives to Animal Use in the Education, Research and Industry held - COLAMA, Niterói, Brasil, no período de 25 a 29 de Novembro de 2012.