

Isolation, Culture and Transfection of Human Skin Keratinocytes: Application in Cell and Gene Therapy in Wound Patients

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ABSTRACT

Human keratinocytes could be used in the repair of damaged skin, in tissue engineering applications, gene therapy and recently, the generation of iPS cells. We isolated human keratinocytes from foreskin and subsequently cultured them on fibronectin, collagen type I, gelatin and laminin-coated dishes that contained three different types of serum-free medium. We developed improved conditions for efficient transfection of these human keratinocytes by testing three common transfection methods and a GFP plasmid vector. The isolated cells showed typical keratinocyte morphology and expressed the epithelial cell specific antigen, cytokeratin 14. Collagen type 1, epilife medium and lipofectamin 2000 gave the best results for isolation and transfection of human keratinocytes. This protocol can be used as a reproducible, simple and efficient method for isolation, cultivation and genetic manipulation of human keratinocytes, which may be useful in cell and gene therapy applications.

Keywords: Isolation, Culture, Transfection, Keratinocyte, Cell Therapy, Gene Therapy