A New Approach for Optimization of Keratinocyte Culture and Fabrication of Keratinocyte Epidermal Sheets without Using 3T3 Feeder Layer

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ABSTRACT

The rise in the incidence of obesity and diabetes has increased the burden of treating chronic wounds. Burn injuries also affect 11 million people worldwide annually. In addition, postsurgical wound cares are not cost effective for healthcare system. Although conventional split-thickness autologous skin grafts (STSGs) are still the gold standard of care for burn treatment, finding an alternative approach is essential due to the disadvantages of skin autografts such as limited healthy donor sites in extensive burns and donor-site morbidity.

Cell therapy can be applied for treatment of various types of skin defects as well as both acute and chronic wounds without major surgical procedures. Following the successful cultivation of keratinocytes by Rheinwald and Green in 1975, these cultured cells were used for treatment of a burn patient for the first time. Keratinocytes consist approximately 90-95% of epidermal cells forming basal, spinous, granular, and cornified layers that correspond to progressive stages of differentiation. In treatment of both chronic and acute wound, reduction of inflammation, induction of cell proliferation and migration, development of angiogenesis and releasing paracrine signaling molecules are some cellular functions accelerating wound healing which are controlled by mesenchymal stem cells. Considering the impacts of MSCs on wound healing, the main aim of this study was the establishment of a novel approach to culture isolated keratinocytes in vitro to generate epidermal keratinocyte sheets without using feeder layer. In

this study the expression of differential (K10, invulocrin, filaggrin) and stem cell (K19, K14, P63 and α6β1 integrin) markers in keratinocytes cultured with a modified medium were examined using flow cytometry and real time PCR on days 7, 14 and 21 and the results were compared with control group (cells cultured with EpiLife medium). The results indicated that this medium may be a good alternative for keratinocyte culture and producing epidermal sheets for therapeutic and clinical purposes.

Keywords: Keratinocyte Culture, Epidermal Sheet, MSC, Wound Healing