EFFECT OF DERMAL FIBROBLAST CONDITIONED MEDIUM ON WOUND HEALING PROPERTIES OF KERATINOCYTES FROM DIFFERENT AGE GROUPS

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1.0 Introduction

Impaired wound healing is accompanied with reduce cell growth and migration due to lack of growth factors and extracellular matrix (ECM) components. Supplementation of growth factors and ECM can be an alternative treatment strategy to restore wound healing potential. Dermal fibroblasts are well known for its ability to secrete various growth factors and ECM components during culture (1) and these secreted factors can be collected as DFCM. Supplementation of DFCM was shown to enhance keratinocytes expansion (2). In this study we evaluated the effect of DFCM from different age groups on in vitro keratinocytes wound healing properties.

2.0 Materials and Methods

Human skin samples (3 samples each for 12-40 and 41-60 years old group) were collected as redundant tissue from normal patients with written consent (FF-2013-232). Skin samples were digested to isolate fibroblasts and keratinocytes as described (2). Fibroblasts from skin sample of a 12-years old patient were cultured until passage 3 (P3), and used to produce DFCM (2). Confluent fibroblasts were incubated with serum free keratinocyte-specific and fibroblast-specific culture medium, and designated as DFCM-KM and DFCM-FM, respectively. Human keratinocytes were harvested from skin samples of two different age groups i.e. 12-40 and 41-60 years old. Wound healing properties of keratinocytes were evaluated via cell attachment, growth rate and wound healing rate. It was found that supplementation of DFCM-KM enhance keratinocyte attachment, while both DFCM increase wound healing rate for both age groups compared to control. These preliminary results suggest that DFCM could be use as potential wound healing mediator.

ARTICLE INFO
Published: 1st December, 2014
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KEYWORDS
Dermal fibroblast conditioned medium, Attachment, Growth rate, Wound healing

ABSTRACT
Dermal fibroblast conditioned medium (DFCM) contains fibroblasts secreted proteins, and had been shown to enhance keratinocytes expansion. The goal of this study was to evaluate the efficacy of DFCM as wound healing mediators for keratinocytes from different age groups. Two different DFCM were prepared using serum free keratinocyte-specific and fibroblast-specific culture medium, and designated as DFCM-KM and DFCM-FM, respectively. Human keratinocytes were harvested from skin samples of two different age groups i.e. 12-40 and 41-60 years old. Wound healing properties of keratinocytes were evaluated via cell attachment, growth rate and wound healing rate. It was found that supplementation of DFCM-KM enhance keratinocyte attachment, while both DFCM increase wound healing rate for both age groups compared to control. These preliminary results suggest that DFCM could be use as potential wound healing mediator.
imaging system. The rate of healing was evaluated as the healing of wounded area in defined culture time.

![Graph showing cell concentration, growth rate, and rate of healing]

**Fig. 1** Effect of DFCM on keratinocytes wound healing properties of cell attachment (A), growth rate (B) and rate of healing (C) for different age group. * indicates significantly different than respective control and # indicates significantly higher between age groups.

### 3.0 Results

As shown in Fig. 1, supplementation of DFCM-KM significantly enhances keratinocyte attachment for 41-60 years old group compared to that of control. Increment in keratinocyte attachment was also observed for >12-40 years old group but not significant compared to control. Supplementation of DFCM-FM reduces keratinocyte attachment for both age groups. In contrast, supplementation of DFCM-KM and DFCM-FM significantly increase wound healing rate for both age groups compared to control. No effect was observed for the growth rate when supplemented with either DFCM.

### 4.0 Discussion

DFCM dependent enhancement of keratinocyte attachment and wound healing was influenced by fibroblast secreted proteins including cytokines, chemokines, growth factors and ECM.(3-4) One-Dimensional protein analysis confirmed that the composition of secreted proteins were different between DFCM-KM and DFCM-FM (data not shown), which may lead to the differential effect on keratinocytes wound healing properties.

### 5.0 Conclusion

DFCM shown to enhance keratinocyte attachment and rate of healing for both age groups, however, comparatively higher enhancement were observed for 12-40 years old group.

**Acknowledgement:**

This study was funded by FF-2013-232 and Science fund 02-01-02-SF0964.

**References**


