CONCENTRATION DEPENDENT EFFECT OF DERMAL FIBROBLAST CONDITIONED MEDIUM ON IN VITRO WOUND HEALING PROPERTIES OF KERATINOCYTES

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ABSTRACT
Fibroblast is known to secrete cytokines, chemokines, growth factors and extracellular matrix (ECM) proteins. These factors can be collected from the waste medium as dermal fibroblast conditioned medium (DFCM). The aim of this study was to evaluate the effect of DFCM on in vitro keratinocyte wound healing properties. DFCM was concentrated using 3kDa centrifugal filter and was supplemented to the serum-free keratinocyte culture medium (EpiLife) at 10, 20 and 40 µg/ml. One-Dimensional SDS PAGE demonstrated the presence of several low and high molecular weight proteins. The efficiency for keratinocyte attachment increased with DFCM but keratinocyte proliferative properties were not affected. Moreover, DFCM at 10 µg/ml showed to increase the in vitro wound healing rate of keratinocytes compared to control and to other concentrations. In conclusion, fibroblast secreted protein in DFCM may promote keratinocytes attachment and migration during in vitro wound healing. This would be of interest in the potential application towards skin injuries.

1.0 Introduction
Fibroblasts secreted proteins can be collected as dermal fibroblast conditioned medium (DFCM). It has been shown to enhance the in vitro expansion and yield of keratinocytes (1, 2). This leads to hypothesis of applying DFCM as potential wound healing mediators in skin injuries, either alone or with acellular 3D skin scaffold for the treatment of acute injuries. In this preliminary study we aimed to evaluate the secretory profile of DFCM and the concentration dependent effect on in vitro keratinocyte wound healing properties.

2.0 Materials and Method
Redundant skin tissues were collected from three normal patients with informed consent (Ethical approval: UKM 1.5.3.5/244/02-01-02-SF0964) and keratinocyte and fibroblasts were harvested according to the protocol describe earlier (2). Fibroblasts cells from a 12-years old patient were cultured until passage 3 to prepare the DFCM using serum-free keratinocyte culture medium (EpiLife), and later concentrated using 3kDa centrifugal filter. Protein
concentration was measured via BCA assay, and protein identification was performed via 1-D SDS PAGE. To evaluate the concentration dependent effect of DFCM, keratinocytes at passage 3 (n=3) were supplemented with DFCM at concentration of either 10, 20 or 40 µg/ml. Attachment efficiency, specific growth rate, migration rate and in vitro wound healing rate were observed using time-lapse imaging and analyzed via image analysis software. Immunostaining with anti-Ki67 was performed to detect the proliferative cells. Images were captured using confocal laser scanning microscopy.

3.0 Results

Figure 1a shows that the proteins concentration for DFCM was higher compared to Epilife. This was confirmed by 1-D SDS PAGE which demonstrated the presence of low and high molecular weight proteins in DFCM (Figure 1b). The efficiency for keratinocyte attachment was significantly increased with DFCM, and at 40µg/ml the value was 1.4 times higher than that of control (Figure 1c). However, no effect was observed on the growth rate and number of proliferative keratinocytes (data not shown). For wound healing rate, DFCM at 10 µg/ml showed an increased in vitro wound healing rate of keratinocytes (1.2 times higher than control), which were significantly higher than other concentrations (Figure 1d).

4.0 Discussions

Results show that supplementation of DFCM enhances keratinocytes attachment and in vitro wound healing rate than
control condition. This indicates that the DFCM may contain higher concentration of proteins most likely ECM components secreted by the fibroblasts.

5.0 Conclusions

Secreted protein in DFCM promotes keratinocytes attachment and migration during healing, indicating the potential application of DFCM in skin injuries.

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References
