Interaction of Electrically Stimulated Fibroblasts with Keratinocytes promotes Better Skin Equivalent Production

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Introduction: Skin grafts are required when the skin is injured with large wounds, burns, or is affected by chronic diseases [1]. Unfortunately, skin autografts are limited by the availability of healthy donor site [1]. Thus, skin replacement therapies, such as skin equivalents (SE), have been developed [1,2]. This study focuses on the SE generated through electrical stimulation (ES). The SE presented in this work includes dermis and epidermis which are respectively formed with fibroblasts and keratinocytes cells [3].

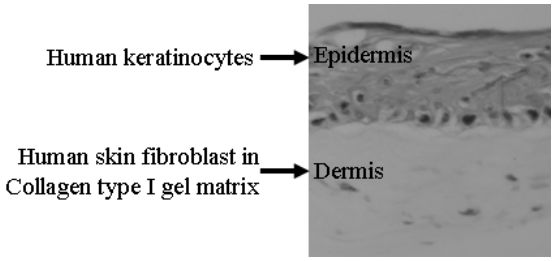

Figure 1. Structure of our skin equivalent

The interaction between the epidermis and the dermis is vital for the skin development. This interaction involves keratinocytes from the epidermis and fibroblasts from the dermis. Our previous studies demonstrated that ES promoted human skin fibroblast growth, enhanced wound healing process and increased the secretion of fibroblast growth factors (FGF1 and FGF2) [4]. The objective of the present study was to investigate the interactions between human keratinocytes and the ES-exposed fibroblasts, and the impact on SE engineering. **Materials and Methods**: Normal human skin fibroblasts and keratinocytes were used in this study. The fibroblasts were cultured in a Dulbecco's modified Eagle's medium, and then used at passages 4 and 5 in the present work. Cells were cultured on the heparin-bioactivated conductive PPy/PLLA membranes which were connected to a DC constant potential source through external electrodes to form a complete circuit. Two potential intensities, 50 and 200 mV/mm, were tested. The cells were exposed to ES for 6 h, and were further cultured for 24 h prior the analyses. Sham ES-exposed control groups followed the same conditions except exposure to ES. Fibroblasts were then collected and mixed with collagen type I to produce dermis. After 2h incubation, human keratinocytes extracted from normal human skin tissue were seeded on the top of the engineered dermis and incubated for 3 days to complete the epidermis. After that, air- liquid interface procedure was performed for another 3 days to allow keratinocyte stratification. Finally the SEs were fixed in a 4% paraformaldehyde solution. The morphology of the SEs was analyzed by histology. The secretion of growth factors, such as keratinocytes growth factor (KGF), by the fibroblasts exposed to ES was also analyzed by ELISA. **Results:** The histology results showed nice keratinocyte stratification giving more cell layers in the ES groups than that in the controls. Furthermore the cohesion between the dermis and epidermis seems better in the skin containing ES-exposed fibroblasts. Interestingly the epidermis was also thicker in the SE prepared by the ES-exposed fibroblasts.

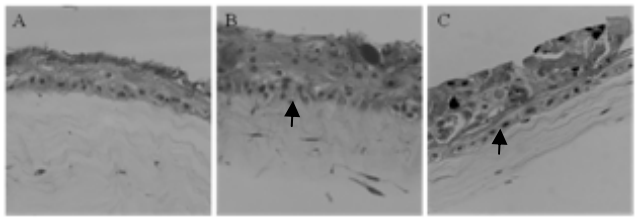

Figure 2. SEs prepared by (A) control, (B) fibroblast exposed to 50mV/mm and (C) fibroblast exposed to 200mV/mm, showing dense basal layer in B and C (arrows).

Additionally, ELISA measurement showed that the level of KGF was higher in the ES-exposed fibroblasts. KGF stimulates keratinocytes proliferation and also enhances differentiation [5]. Thus, ELISA results support the histological observations

Conclusion: The structural analyses demonstrated morphological differences between the SEs prepared by the ES-exposed and control fibroblasts. The better stratified layers in the ES-exposed SEs could be linked to the higher level of KGF secretion in human skin fibroblast cells exposed to ES.

References:

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