Mechanical Conditioning of Keratinocyte Monolayers

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Introduction

Tissue engineered skin replacements have opened new options to treat skin loss. Although increased healing rates of burn and/or chronic wounds can be observed with those engineered constructs, several intrinsic shortcomings still limit their use, including inadequate adhesiveness and mechanical properties, and sub-optimal manufacturing processes (duration, costs). In this context, bioreactors could be the key to accomplish reliable, traceable and cost-effective tissue engineered processes, with minimal risks of infection. Moreover, stretching proved to be a key stimulus for cell growth, differentiation and migration, tissue remodeling and gene expression [1,2].

The aim of the present study is to investigate the role of mechanical conditioning in keratinocyte monolayer cultures using our previously developed bioreactor (Figure 1). Cell viability and morphology, and matrix metalloproteinases (MMP) 2, 9 and 28 – shown to be implicated in cell migration, proliferation and tissue remodeling [3] – were evaluated in dynamically cultured constructs and static controls.

Materials and Methods

Immortalized human keratinocytes were seeded at 1x10^6 cells/ml (3 drops, 15µl each) on commercial silicone sheets (5x50mm, Specialty Manufacturing Inc, USA) coated with type I collagen and confluent monolayer was obtained after 24 hours. Two different stretching patterns were applied to the cell-scaffold constructs: as to the first, a 10% deformation is maintained for 2 hours and 55 minutes (relaxation); as to the second, a sequence of stretching, resting and cyclic stretching phases is imposed (cyclic deformation). For each experiment, twelve samples were used: four for static culture, four for the relaxation protocol and four for the cyclic deformation one. Investigational procedures were performed at 0, 24, 48 and 72 hours after stimulation. Cell viability was measured using the TOX-8 (Sigma Aldrich) enzymatic colorimetric assay. For morphological assessment, keratinocytes were stained with 0.01% toluidine blue in water and changes at the drops edges were observed at 10X magnification. The expression of MMP 2, 9 and 28 was analyzed by RT-PCR (Promega) and quantified using ImageJ software (http://rsb.info.nih.gov/ij/); expression levels were normalized upon beta-actin signals. Gelatinase activity of both MMP-2 and MMP-9 was analyzed by zymography.

Results

The results of the cell viability tests suggest that cellular proliferation is not affected by the relaxation pattern, whereas a decrease is registered after cyclic deformation stimuli. Morphological analysis showed proliferating and migrating cells in all constructs, and a multiple layers organization at the drops edges in most relaxation samples at later time-points. The expression of MMP-2 and MMP-28 is increased in all the stimulated constructs. The expression of MMP-9 is nearly maintained at 72h in all the samples. The relative change in gene expression between control and stimulated groups was however modest at each time-point. Zymography analysis showed a strong gelatinase activity for the investigated MMPs at all time-points, with no significant differences between samples subjected to different stimulation patterns. Further studies are currently in progress to strengthen the collected data and to investigate the effects of longer stimulation period (t=24, 48h).

Discussion and Conclusions

The present study confirmed our bioreactor as a safe, reliable and effective device to investigate the role of mechanical conditioning in keratinocytes monolayer cultures. The collected results show that dynamic stimulation directly affects both the cellular growth pattern of keratinocytes monolayers and the modulation of some proteolytic enzymes involved in cellular migration, proliferation and in tissue remodeling. Our work thus confirms the effectiveness of the dynamic culture approach in skin tissue engineering and represents a useful preliminary study to identify the optimum stimulation pattern to improve the in vitro engineering of skin substitutes.

References


Authors have nothing to disclose.