0236 Unravelling the path to create a cell sheet-based model of skin scar-like tissue

Helena Moreira[1,2], Daniel Rodrigues[1,2], Rui Reis[1,2], Alexandra Marques[1,2]

[1]3B's Research Group – Biomaterials, Biodegradable and Biomimetics, Guimarães, Portugal, [2]ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

Regardless of the advances in understanding the mechanisms and the pathophysiology behind skin deformities, scaring continues to be an unsolved clinical problem. The underlying wound healing process involves a series of key cells which play different key roles. Fibroblasts are known to suffer the influence of local biochemical (e.g TGF-β1) and biomechanical signaling upon a wound scenario leading to a phenotypical change into myofibroblasts. The latter enhance immature extracellular matrix (ECM) synthesis and generate tensional forces that leads to ECM reorganization. Certain skin pathologies (e.g hypertrophic scars) rise from a dysfunction of this underlying regulatory mechanism which in turn drives myofibroblast persistence in the wound. When trying to study the mechanisms behind scarring human ex vivo samples are many times scarce and most of the current in vitro systems rely on standard 2D cultures of keloid/hypertrophic scar fibroblasts. Taking all of this into consideration we propose the use of cell sheet technology to create an *in vitro* 3D scar model. Herein we report the effect of TGF-β1 in human dermal fibroblast cell sheets as the first step to attain cell sheets with a myofibroblast-like phenotype in which cells are embedded in a scar-like ECM. To further strengthen our concept we performed the stacking of pre-formed cell sheets generating a cohesive 3D scar-like tissue.

Human dermal fibroblast (hDFbs) cell sheets were produced as previously described[1], and stimulated with TGF-β1 (10ng/ml) over 7, 14 and 21 days. Following phenotype and ECM characterization, cell sheets were stacked in order to obtain a 3D structure composed of 2 or 3 cell-sheets. The analysis of key genes (q-PCR) and proteins (Western blot and immunocytochemistry) showed that hDFbs cell sheets, when stimulated with TGF-β1 present an increased expression of α-SMA, fibronectin (FN) ED-A and FN ED-B, characteristic of a myofibroblast-like phenotype. When looking into the expression of scar ECM-associated proteins, hDFbs cell sheets obtained in the presence of TGF-β1 produced higher amounts of fibronectin and collagen I. Stable 3D constructs with a noticeable level of integration after a total of 21 days of culture, were further created upon stacking of the cell sheets obtained after 7days of culture in the presence of TGF-β1.

In conclusion, this work suggested that it is possible to promote the secretion of scar-like ECM in hDFbs cell sheets due to phenotypic changes into myofibroblast-like cells when stimulated with TGF-β1. Cohesive 3D scar-like tissue structures were obtained which opens the possibility to develop a highly accurate *in vitro* 3D scar model to study underlying cellular mechanisms involved in the wound healing deregulation.

Reference: [1] M.T. Cerqueira, et al. (2014). Acta Biomaterialia 10(7): 3145-3155.

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