WOUND HEALING

IN VITRO ENGINEERING OF A SKIN SUBSTITUTE BASED ON ADIPOSE-DERIVED STEM CELLS AND HUMAN KERATINOCYTES

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Background: In the field of wound healing, stem cell-based strategies are gaining importance for their regenerative potential. Mesenchymal stem cells (MSCs) are considered today the best candidate for their relative abundance and accessibility in the human body. Adipose-derived stem cells (ADSCs) are a particular subset of MSCs present in the stromal-vascular fraction of the adipose tissue.

Objective: The goal of our research was to isolate ADSCs from the stromal-vascular fraction of adipose tissue and to demonstrate their in-vitro capability to give rise to cellular dermal scaffolds promoting cell migration and proliferation, wound healing and tissue regeneration.

Materials and methods: First, we characterized ADSCs, both for the expression of specific markers on their surface and their pluripotency. Then we assessed extracellular matrix (ECM) production by histochemical staining and immunofluorescence analysis. We also performed a wound-healing assay, thereby demonstrating ADSC-mediated production of soluble factors promoting cell motility and proliferation. We seeded keratinocytes on ADSC-induced dermal scaffolds and we assessed the expression of molecules crucial for cell-ECM interactions (such as collagen I and IV, fibronectin and integrin αV).

Results: ADSCs were defined as CD90, CD73 and CD105 positive cells. Their pluripotency was proven through adipogenic and osteogenic differentiation assays. ADSCs turned out to be capable of gaining a fibroblast-like phenotype and efficiently producing a collagen and fibronectin-containing dermal matrix upon stimulation with ascorbic acid. The scratch test showed ADSC-conditioned medium was able to promote re-epithelialization. Moreover, keratinocytes were efficiently seeded on the ADSC-induced matrix, thus confirming the regenerative potential of ADSC.

Conclusions: The scaffolding material directly produced by ADSCs has several advantages: it supports cell-ECM interaction and can be easily colonized by surrounding other MSCs, keratinocytes, fibroblasts, endothelial cells and their precursors. Moreover, it can be directly grafted with keratinocytes layers, thus by-passing the classical two-step grafting procedure.