Skin-derived Cell-sheets as Powerful Tools to Engineer Skin Analogues

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Cell/cell-extracellular matrix (ECM) dynamic interactions appear to have a major role in regulating communication through soluble signaling, directing cell binding and activating substrates that participate in the highly organized wound healing process. Moreover, these interactions are also crucial for *in vitro* mimicking cutaneous physiology. Herein we explore cell sheet (CS) engineering to create cellular constructs formed by keratinocytes (hKC), fibroblasts (hDFB) and dermal microvascular endothelial cells (hDMEC), to target skin wound healing but also the *in vitro* recreation of relevant models. Taking advantage of temperature-responsive culture surfaces, which allow harvesting cultured cells as intact sheets along with the deposited native ECM, varied combinations of homotypic and heterotypic three-dimensional (3-D) CS-based constructs were developed. Constructs combining one CS of keratinocytes as an epidermis-like layer plus a vascularized dermis composed by hDFB and hDMECs were assembled as skin analogues for advancing *in vitro* testing. Simultaneously both hKC and hDMEC were shown to significantly contribute to the re-epithelialization of full-thickness mice skin wounds by promoting an early epithelial coverage, while hDMEC significantly lead to increased vessels density, incorporating the neovasculature. Thus, although determined by the cellular nature of the constructs, these outcomes demonstrated that CS engineering appear as an unique technology that open the possibility to create numerous combinations of 3D constructs to target defective wound healing as well as the construction of *in vitro* models to further mimic cutaneous functions crucial for drug screening and cosmetic testing assays.

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