494h Transcriptional Profiling of Engineered Skin: the Role of Air-Liquid Interface on Epidermal Development and Stratification

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The human epidermis is a highly differentiated tissue composed of multiple cell layers. The basal cell layer is composed of the stem cells that give rise to more differentiated cells at a controlled rate. These cells move up and start an intrinsic program of differentiation. The outcome of this program is the assembly of epithelial tissue composed of spinous, granular and cornified layers. Each layer displays unique morphological and biochemical properties as a result of a well-orchestrated dynamic process of gene regulation that results in differentiated tissue phenotype. We employed the affymetrix whole genome expression arrays to identify genes that play a key role in the development of engineered skin. This gene chip contains probe sets for analysis of over 47,000 transcripts, covering the whole human genome. Our model system uses de-epidermalized human dermis as substrate for keratinocyte growth. The processed dermis retains many of its structural elements (e.g. laminin, collagen IV) and does not invoke a detectable immune response in animals. Human foreskin keratinocytes were placed on acellular dermis and grown submerged under culture medium for three days, before they were raised to the air-liquid interface. When cultured at the air-liquid interface the engineered tissues differentiated and form a well-stratified epidermis, with all basal, spinal, granular and cornified layers. In contrast, tissues grown under submerged conditions did not exhibit a fully differentiated epithelium, failed to make stratum corneum and lacked barrier function. To understand the role of the air-liquid interface in epidermal development we compared the gene expression profile of submerged tissues with that of tissues grown at the air-liquid interface at different times of development. We identified multiple genes that participate in the development of three-dimensional skin epithelium. Most importantly we identified genes that were not previously known to be important in the process of epidermal morphogenesis. The micro-array results were verified using real time PCR and at the protein level using histology and immuno-histochemistry. Our studies provide molecular level information that may help us develop novel design criteria for the preparation of tissue substitutes that closely resemble natural tissues. Finally, our data suggests that functional genomics may be used in tissue engineering to understand tissue development, wound regeneration and response of engineered tissues to environmental stimuli.