

ON THE POSSIBILITY TO BIOENGINEER SKIN ON CHICK CHORIOALLANTOIS

¹A. Della Vedova, ¹P. Blasi, ²M. Andreassi, ³L. Barberini, ⁴P.F. Alberti, ⁵G. Mariotti, ⁵M. Fimiani, ³C.

Cirotto

¹Dip. Chimica e Tecnologia del Farmaco, Università degli Studi di Perugia; ²Dip. Biotecnologie Chimica Farmacia, Università di Siena; ³Dip. Biologia Cellulare ed Ambientale, ⁴Dip. Medicina Sperimentale e Scienze Biochimiche, Università degli Studi di Perugia; ⁵Dip. Scienze Dermatologiche, Università di Siena.
e-mail: dv.alessia@hotmail.it

Purpose. To evaluate the possibility to engineer skin on the chicken chorioallantoic membrane (CAM) starting from de-cellularized de-epidermized human dermis (DED) [1] and immortalized keratinocytes with the aim of replacing animals in short-term investigations in dermatology.

Methods. Immortalized human keratinocytes (Hacat) [2] were cultured on DED for 3 days. Fertilized chicken eggs were incubated and, on day 7, circular DED samples (6 mm) were implanted on the CAM after provoking an ectodermal epithelium lesion with a teflon punch. On day 12 the xenografts were excised together with the surrounding CAM, the tissue fixed in 4% paraformaldehyde, paraffin-embedded and cut with a microtome to obtain 5-10 μ m slides. Staining was performed with hematoxylin and eosin. Alternatively, whole dehydrated samples were imaged by scanning electron microscopy (SEM).

Results. Already 2 days after transplantation, the skin grafts were invaded by the CAM erythrocytes and neo-endothelium was present. The presence of nucleated chick erythrocytes confirmed that the DED was nourished by the host blood. Epidermal growth factor (EGF) [3] accelerated and improved neoangiogenesis in the CAM and implanted DED. On day 12, SEM observation of the DED cultured with Hacat revealed keratinocyte stratification and the initial development of a multilayered epithelium.

Conclusion. Our results indicate that it is possible to use the revascularized DED to support the formation of an epidermis provided with stratum corneum. The future goal of this project is to establish the epidermal barrier using primary epidermal cells and test the barrier function by examining the permeability to various chemical compounds.

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[2] Boelsma E, Verhoeven MC, Ponc M, Reconstruction of a human skin equivalent using a spontaneously transformed keratinocyte cell line (HaCaT), *J Invest Dermatol.* 1999; 112(4): 489- 498.

[3] Akimoto Y, Obinata A, Endo H, Hirano H, Epidermal growth factor (EGF)-induced morphological changes in the basement membrane of chick embryonic skin, *Cell Tissue Res.* 1988; 254: 481-485.