Applications for in vitro reconstructed epidermis with melanocytes

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Introduction

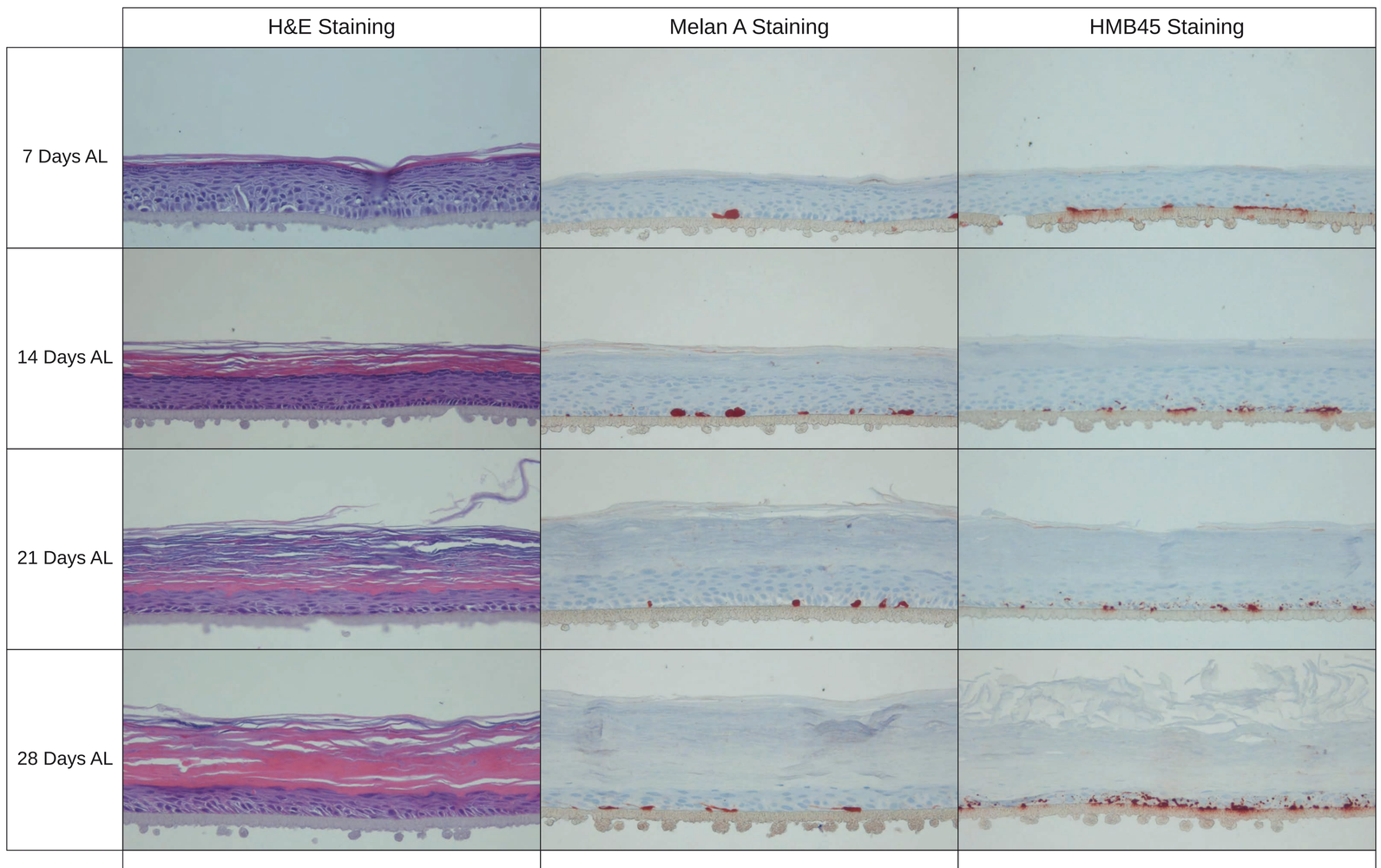
CellSystems' in vitro reconstructed epidermis with melanocytes (epiCS-M) is a human pigmented 3D co-culture system consisting of normal human epidermal keratinocytes and melanocytes which can be cultured at the air liquid interface (AL) for up to 4 weeks. This highly differentiated model of the human epidermis shows epithelial stratification and cornification in the upper layers with an intact barrier function while the melanocytes and proliferating keratinocytes are located in the basal compartment. Barrier function and tissue viability are reproducibly high. Melanogenesis can be observed throughout the culture period. The pigmented epidermis model can be varied using melanocytes from Caucasian, Asian-Caucasian or Afro-American donors (which results in different degrees of pigmentation). The pigmented epidermis model epiCS-M is an ideal tool for skin tanning or bleaching studies as well as for melanogenesis research.

epiCS-M Model Characterisation

The reconstructed human pigmented epidermal model is characterised by a fully differentiated stratified epidermis where melanocytes are located in the basal cell layer. Melanin is synthesized by melanocytes and transferred to the neighbouring keratinocytes by their dendrites. To further characterize this in vitro skin model we investigated the tissue morphology for up to 4 weeks starting on day 7 AL. Melan A and HMB45 stainings show the location of melanin and melanocytes in the basal layers of the model during the airlift culture period between day 7 and day 28. As an example a lightly pigmented epidermis containing melanocytes derived from an Asian-Caucasian donor is shown.

Protocol of skin tanning experiments

- After arrival of epiCS-M tissues experiments should start best after a 2 - 20 hours adaption phase
- Dilute a stock solution (e.g., IBMX 50 mM in DMSO) to the final concentration in epiCS-M culture medium
- Ensure that the final DMSO concentration does not exceed 0,1 %
- Aspirate the regular epiCS-M culture medium and replace it with 2 ml freshly prepared medium containing the test substance (37 °C) for each well (6-well format)
- Culture the epidermis equivalents in the incubator (37 °C, 5 % CO_2, 95 % RH) for two days
- Remove the old medium and replace it with 2 ml new epiCS-M culture medium containing the test substance (37 °C) for each well
- Change medium every other day up to 2 weeks



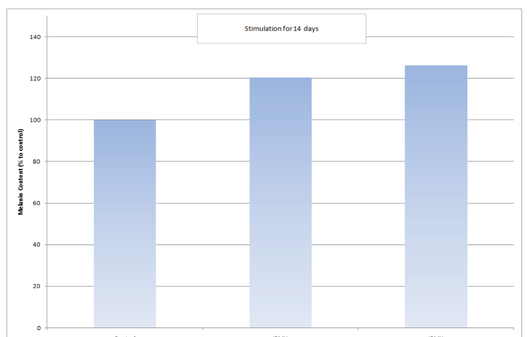
H&E:
Standard Hematoxylin/Eosin staining demonstrates the development of the stratum corneum between day 7 and day 28 of airlift culture.

Anti-Melan A Antibody:
Melan A recognises an antigen present in organelles and small vesicles dispersed over the entire cytoplasm of the melanocytes. Melanocytes are located in the basal layers of the tissue.

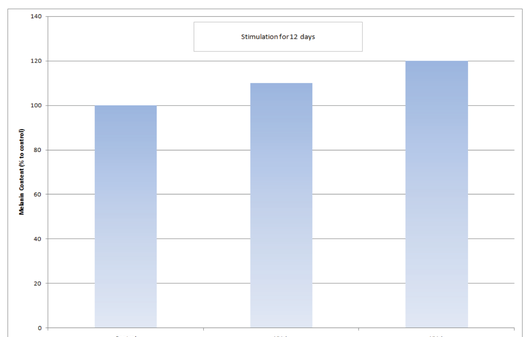
Anti-Melanoma Antibody:
The HMB45 antibody reacts with a glycoconjugate present in melanosomes. Non-melanocytic cells are negative. Melanocytes are located in the basal layers of the tissue.

Modulation of Melanogenesis

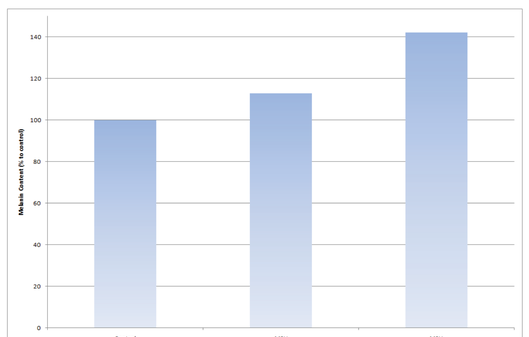
To study the tanning potential of the model we stimulated the melanin production with UV light (30 and 45 mJ/cm^2), IBMX (3-Isobutyl-1-Methyl-Xanthine), or α-MSH (Melanin Stimulating Hormone). Bleaching of tanned tissue models was achieved with Kojic Acid (5-hydroxy-2-(hydroxymethyl)-4-pyrone), a known inhibitor of melanogenesis and therefore used for whitening in cosmetic products. The melanin was extracted from the tissues by incubation of the epidermis in 360 µl solvable (Perkin Elmer) at 100 °C. The optical density was determined at 492 nm wavelength to calculate the melanin content of single epiCS-M tissues.



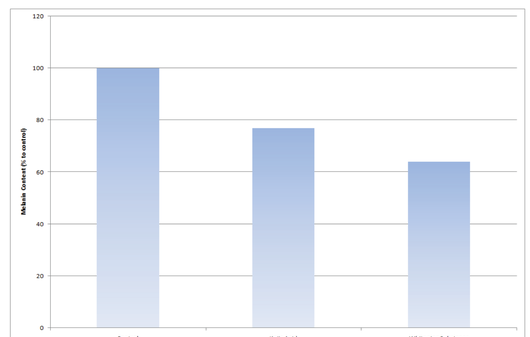
IBMX stimulation:
Exposure for 14 days to 3-Isobutyl-1-Methyl-Xanthine (IBMX, 50 µM systemically applied), which is also known to stimulate melanogenesis, yielded an increased melanin content in the tissues.



UV-A Stimulation:
Repeated exposure to UV light (30 and 45 mJ/cm2) for 12 days resulted in increased melanin production.



Alpha MSH stimulation:
Application of alpha-MSH (Melanin Stimulating Hormone, 100 nM systemically applied) for up to 21 days induced a significant increase of melanin production.



Bleaching experiments:
Treatment with Kojic Acid (5-hydroxy-2-(hydroxymethyl)-4-pyrone, 1% topically applied) or a Whitening Test Substance (systemically applied) resulted in a significant decrease of both the visible pigmentation and the melanin content

Conclusions

The reconstructed epidermis model with melanocytes epiCS-M is a ready to use commercially available in vitro model of the human epidermis where melanocytes are located within the basal cell layer. Due to the skin barrier function of the epidermis liquid, creamy and solid substances can be applied topically - onto the stratum corneum - to closely mimic the in vivo situation of cosmetics application. We showed that some substances can also be applied systemically by adding to the cell culture medium. Both ways of application allow pigmentation studies (i.e. skin tanning or skin bleaching). Pigmentation can also be stimulated by irradiating epiCS-M with UV light.
epiCS-M can be cultured up to 28 days at airlift culture (AL) thereby providing a time window of 2-3 weeks to study pigmentation or de-pigmentation. It also allows to investigate skin differentiation and barrier formation over a substantial period of time.