Modulation of pigmented epidermis models in vitro: epiCS®-M

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Introduction

The *in vitro* skin model epiCS-M comprising keratinocytes and melanocytes is routinely manufactured by CellSystems® for use within a wide range of applications. The main task of melanocytes is the production of melanin and its transfer to the keratinocytes thus inducing skin pigmentation. Moreover, secretion of growth factors by keratinocytes stimulates proliferation and differentiation of melanocytes. The macroscopic appearance of the pigmented epidermis model can be varied using melanocytes from Caucasian, Asian-Caucasian or Afro-American donors resulting in different degrees of pigmentation (**Fig. 1 and 2**). For various investigations it might be of interest to establish a model combining two cell types from the same ethnicity (e.g., Caucasian-Caucasian or Asian-Asian) or any combination of interest. Modifying the basal rate of pigmentation by the use of special culture media might increase the dynamic range of cellular responses in pigmentation and depigmentation studies (**Fig. 3**).

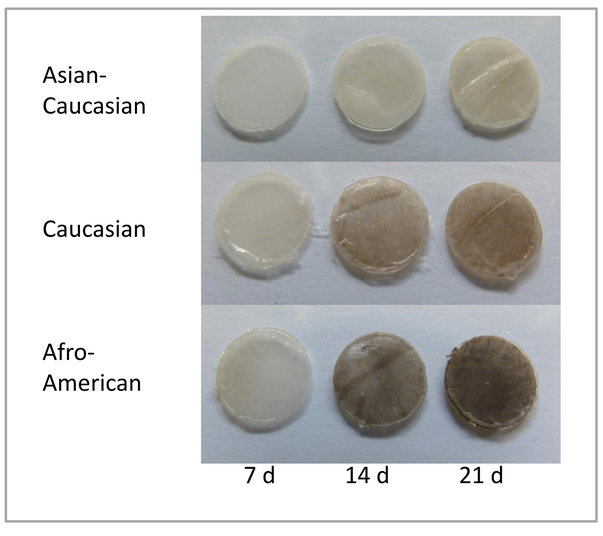


Fig. 1
The melanogenesis can be observed throughout the culture period. Pigmentation increases over time from bright to dark. Three melanocyte donors displaying different intensities during 3 weeks of airlift culture.

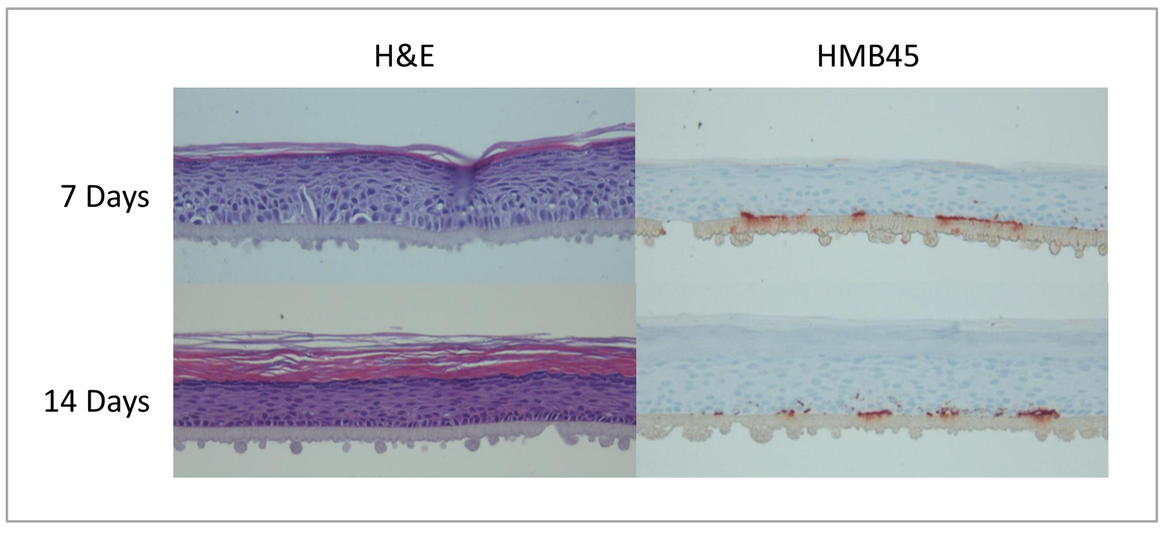


Fig. 2
Left: Hematoxylin/Eosin staining demonstrates the development of the stratum corneum. Right: HMB45 reacts with a glycoconjugate present in melanosomes and shows the location of melanin and melanocytes in the basal layers of the model.

Results

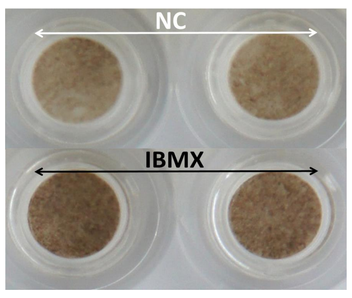


Fig. 3A
Standard medium

IBMX increases pigmentation in epiCS-M by ~50% via an unspecific mechanism.

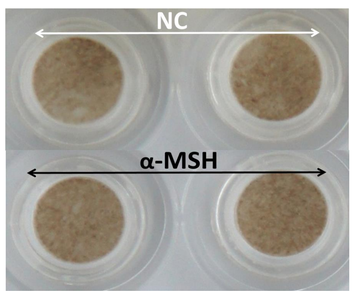


Fig. 3B
Standard medium

α-MSH increases receptor mediated pigmentation by ~10%, which is hardly visible.

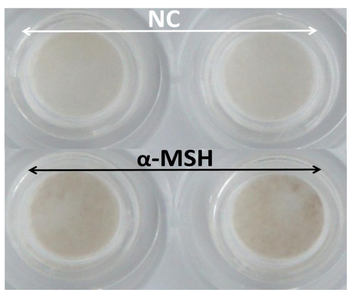


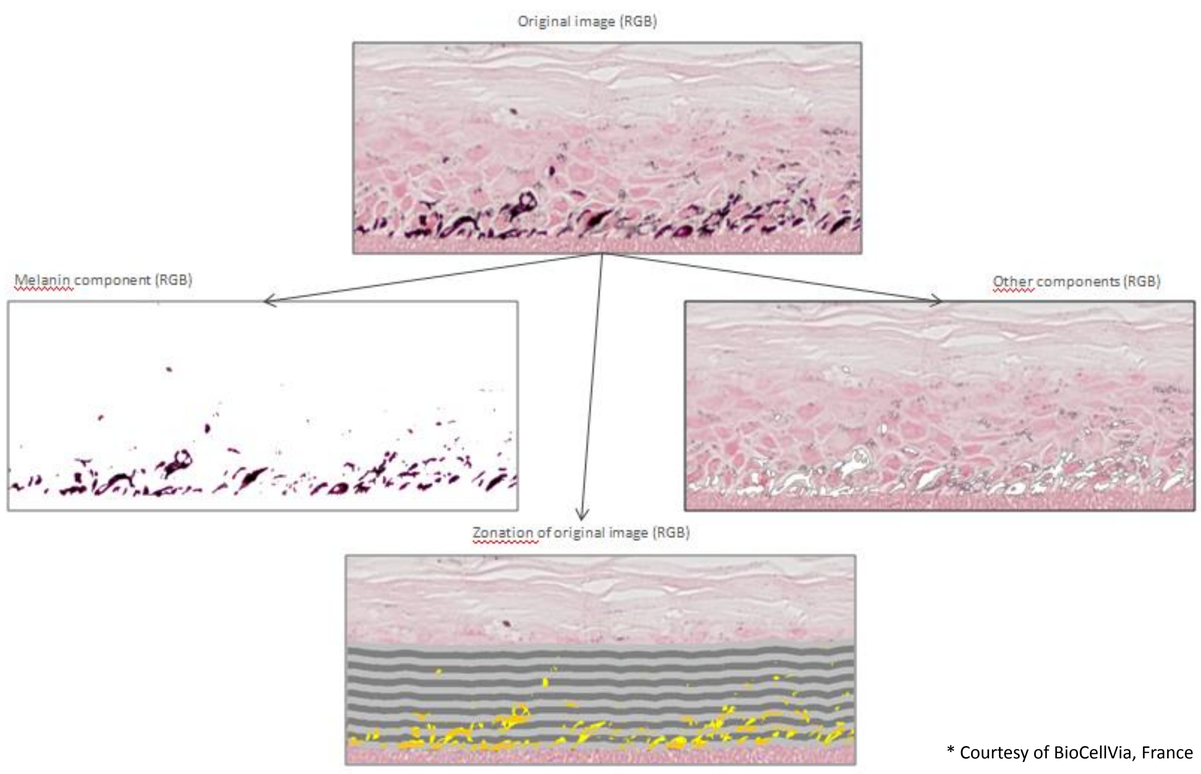
Fig. 3C
LowTan medium

α -MSH increases receptor mediated pigmentation by ~25%. This effect is clearly visible in LowTan medium.

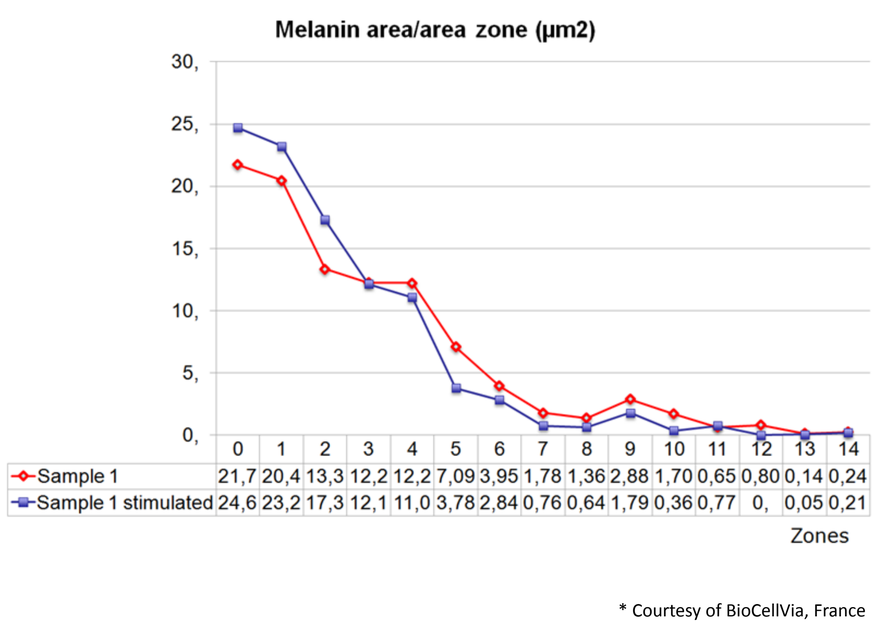
In standard culture medium melanogenesis is stimulated with IBMX (3-Isobutyl-1-Methyl-Xanthine), a substance that reduces degradation of cAMP, by about 50%, when compared to the negative control (**Fig. 3A**). Using α-MSH a weak stimulation (10%) occurs, but is hardly visible due to high basal melanin content (**Fig. 3B**). Reducing the basal rate of pigmentation by the use of a LowTan culture medium increases the dynamic range of cellular responses with regard to the pigmentation degree of the model. **Fig. 3C** shows a tanning example following α-MSH stimulation using the LowTan culture medium. Pigmentation increases about 25% compared to the respective negative control.

Test system: epiCS-M with Caucasian melanocytes after 4 weeks of airlift culture.
Melanin quantification: Standard extraction of melanin with Solvable and subsequent photometric quantification at 492 nm wavelength.

Quantification of melanogenesis in standard medium by digital image analysis*



* Courtesy of BioCellVia, France



* Courtesy of BioCellVia, France

Method: Fontana-Masson (F-M) staining of melanin and nuclear fast red counterstain in a deparaffinized tissue slice. Quantification of black areas (melanin) in different zones of the tissue. Comparison of melanin content in untreated and α-MSH stimulated tissues.

Results: Analysis of F-M staining in 5 bottom zones revealed a 10% increase of melanin content due to α-MSH stimulation in standard medium.

Conclusions

The dynamic range of the pigmentation degree in epiCS-M can be increased by reducing the basal pigmentation rate with a LowTan culture medium. An increase of receptor-mediated tanning with the MCR1 agonist α-MSH from 10% to 25% can be observed with this method. Moreover, digital image analysis of Fontana-Masson stained tissue slices can be used to quantify the melanin production and pigmentation as an alternative to standard procedures like melanin extraction with Solvable. Due to its robustness the epiCS-M reconstructed epidermis model allows long term studies for efficacy testing of tanning and whitening substances, melanogenesis or tissue maturation.