



# Usefulness of in vitro reconstructed pigmented epidermis comprising melanocytes

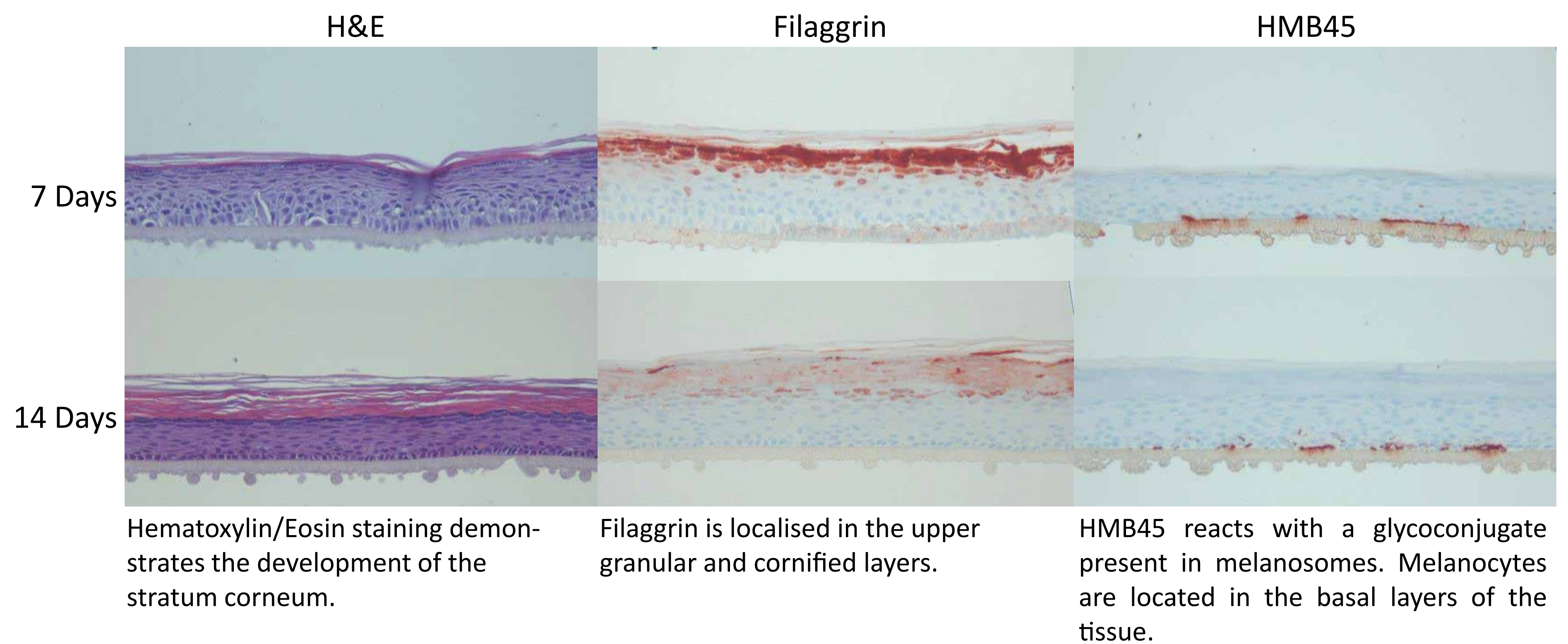
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## Introduction

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

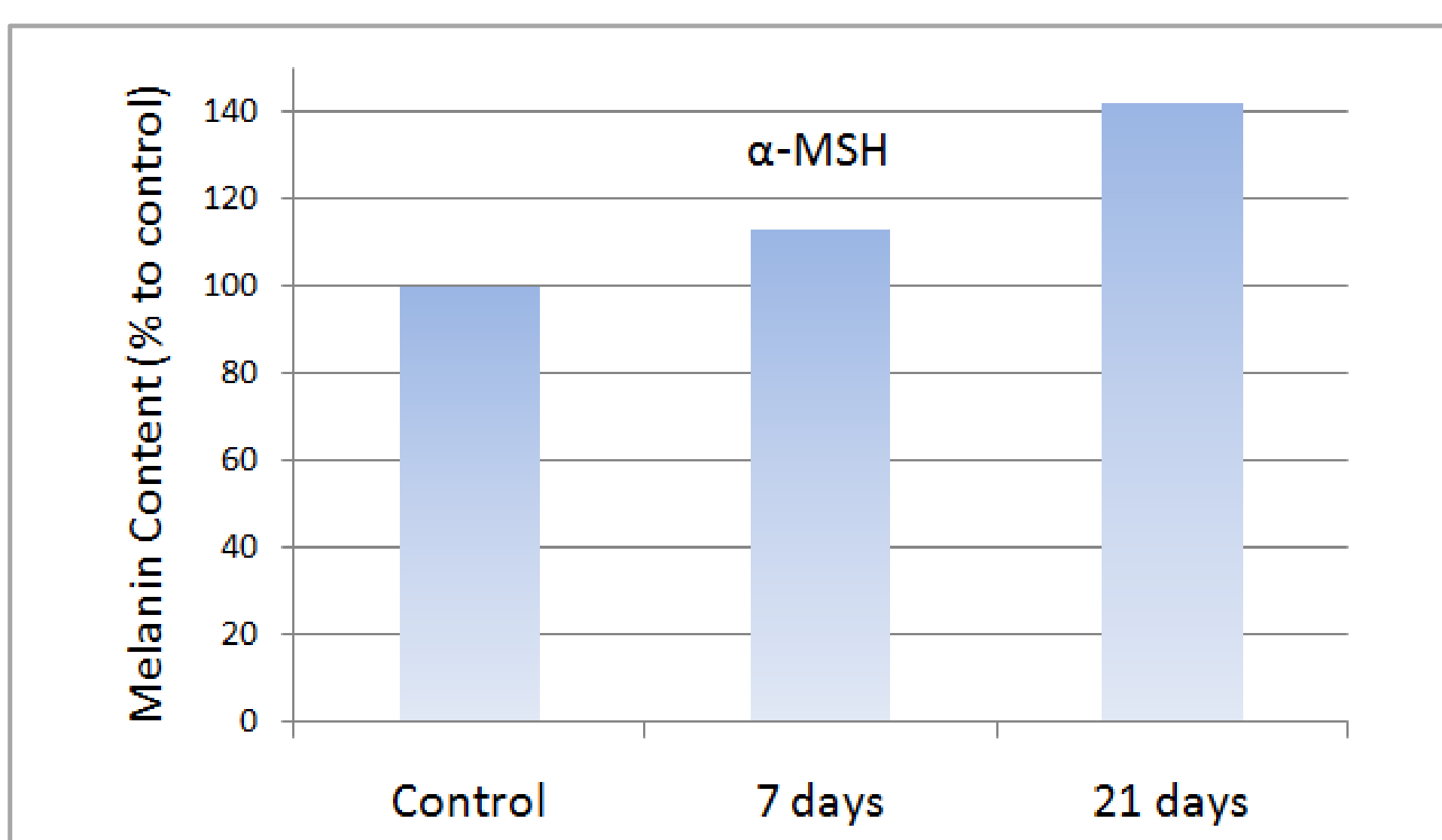
The reconstructed human pigmented epidermal model is characterised by a fully differentiated stratified epidermis where melanocytes are located in the basal cell layer. Melanin is synthesized by melanocytes and transferred to the neighbouring keratinocytes by their dendrites. To further characterize this in vitro skin model we investigated the tissue morphology for up to 4 weeks starting on day 7 AL. HMB45 stainings show the location of melanin and melanocytes in the basal layers of the model during the airlift culture period.



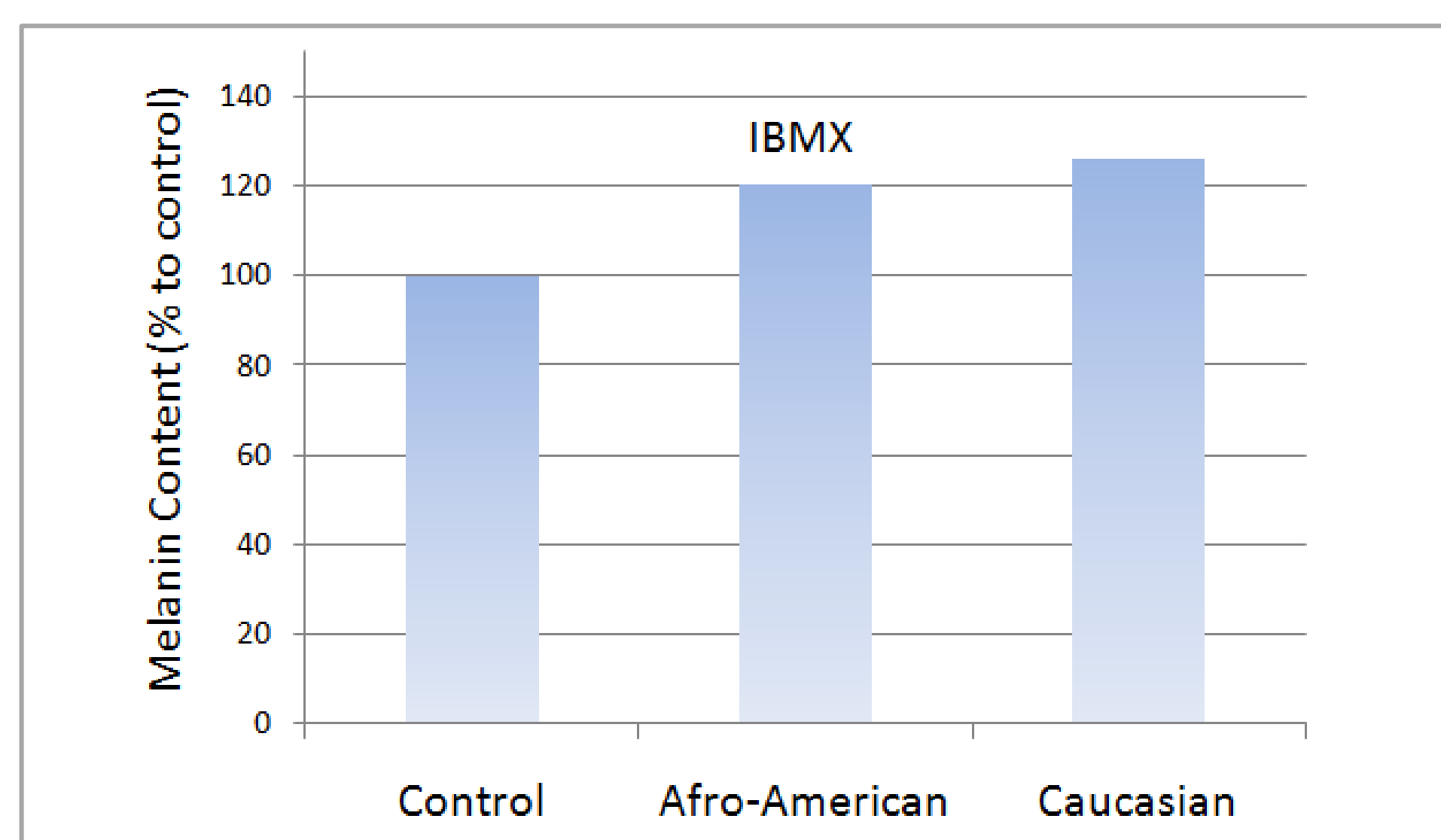
## Modulation of Melanogenesis

### Tanning:

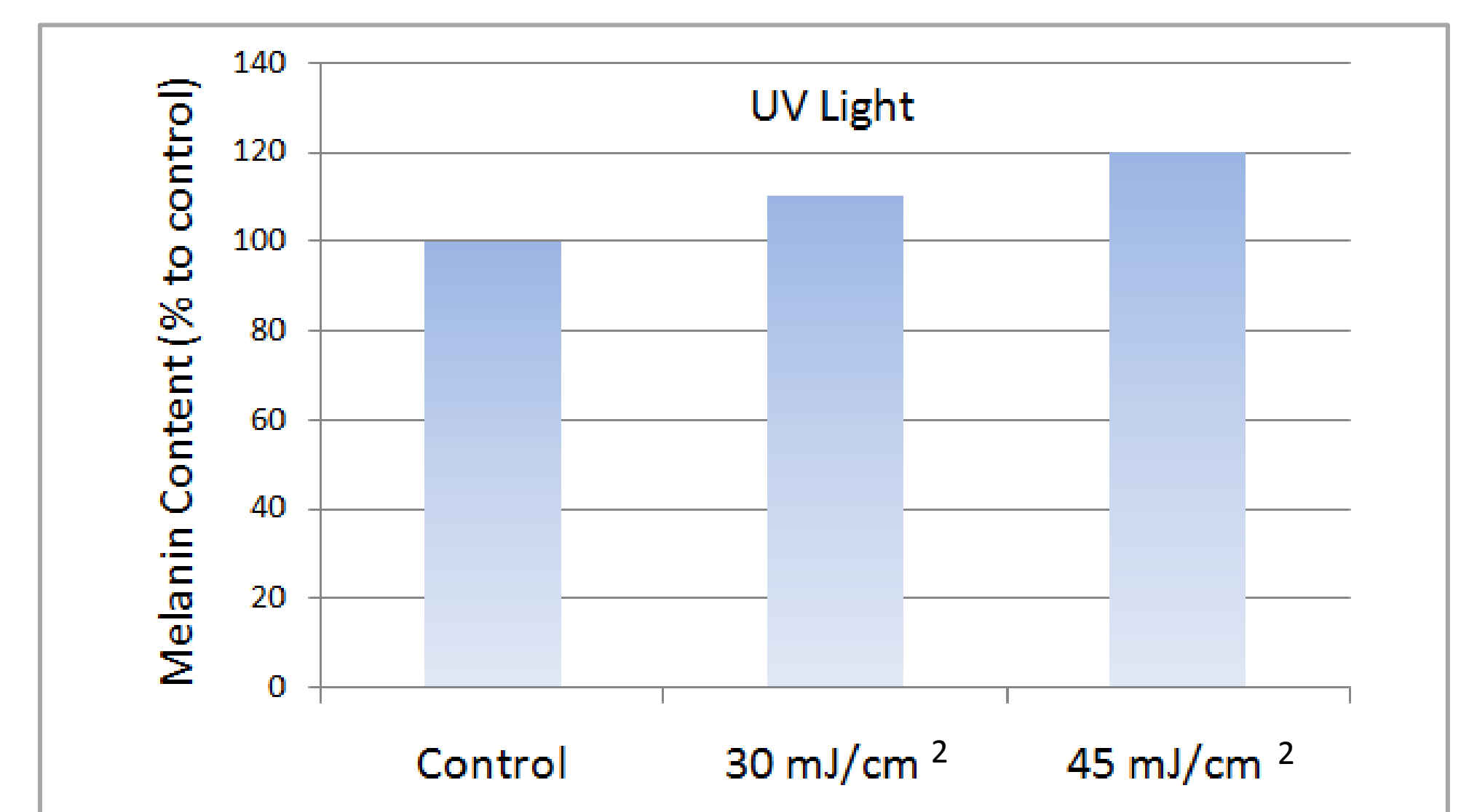
To study the tanning potential of the model we stimulated the melanin production with UV light (30 and 45 mJ/cm<sup>2</sup>), IBMX (3-Isobutyl-1-Methyl-Xanthine), or  $\alpha$ -MSH (Melanin Stimulating Hormone). The melanin was extracted from the tissues by incubation of the epidermis in 360  $\mu$ l 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.



$\alpha$ -MSH, 10 nM, systemically applied



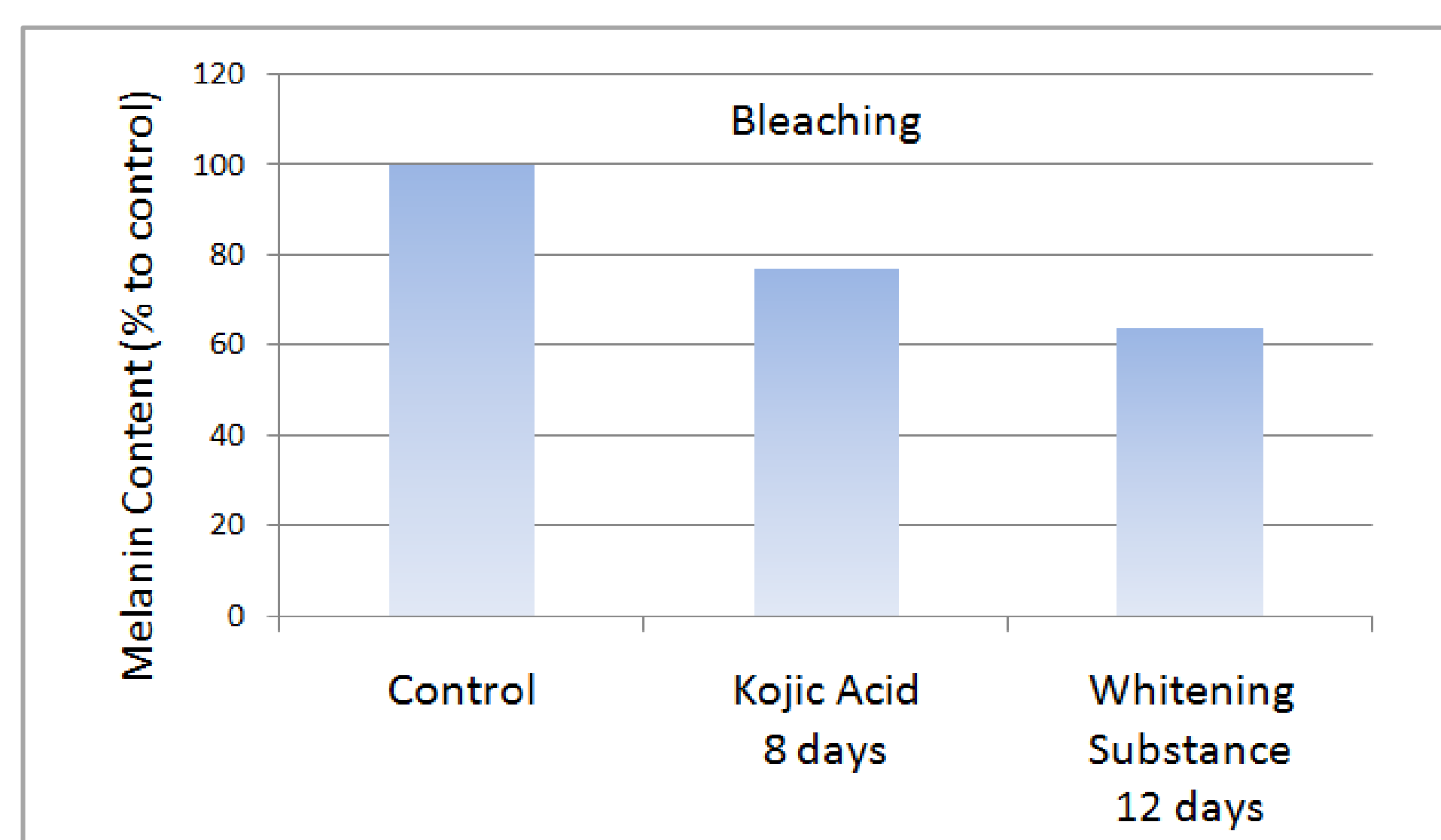
IBMX, 50  $\mu$ M, systemically applied, 14 days



UV light, repeated exposure, 12 days

### Bleaching:

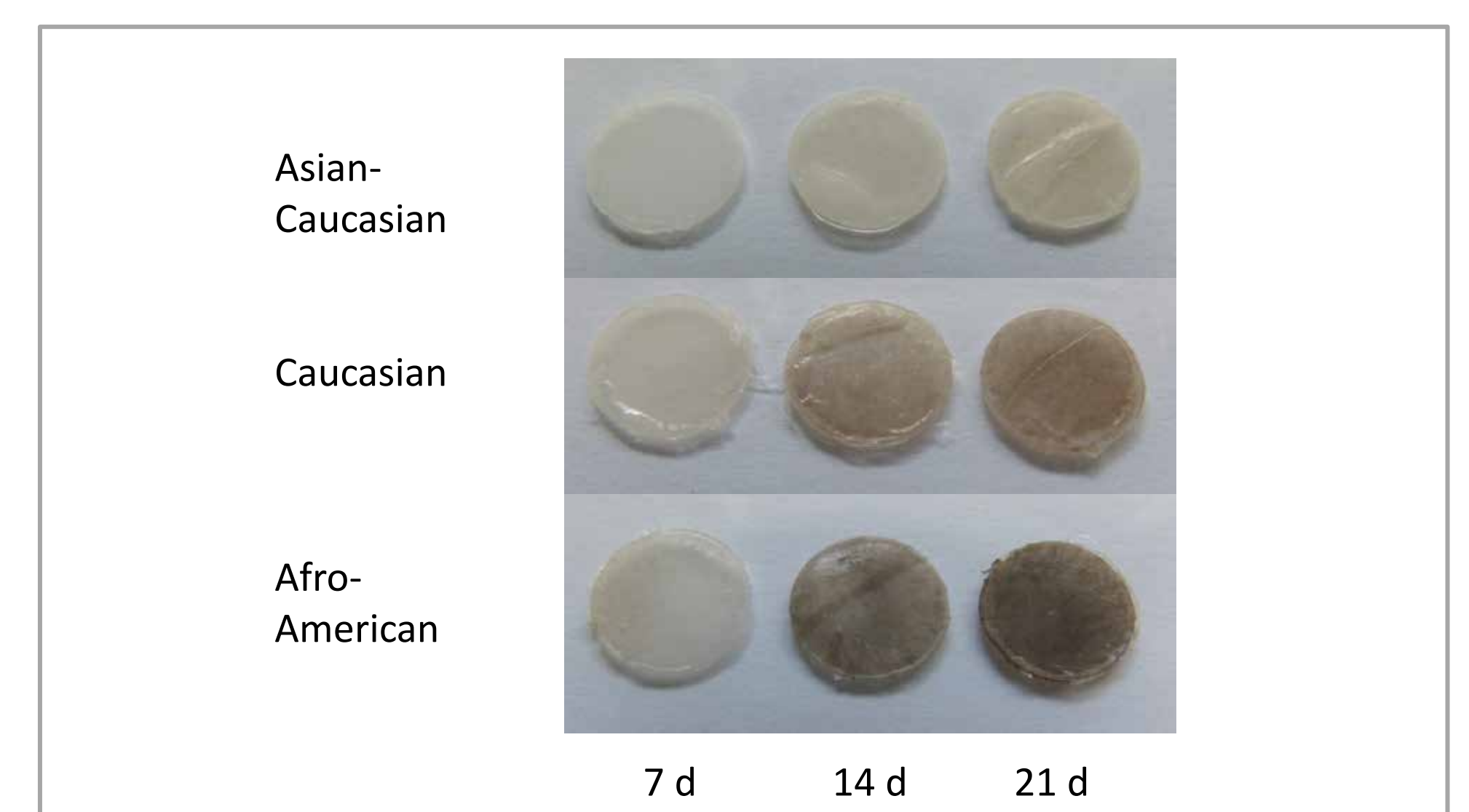
Bleaching of tanned tissue models was achieved with Kojic Acid (5-hydroxy-2-(hydroxymethyl)-4-pyrone), a known inhibitor of melanogenesis and therefore used for whitening in cosmetic products.



Kojic Acid, 1%, topically applied and a Whitening Test Substance, systemically applied

### Maturation:

Development of melanogenesis. Three donors displaying different stages during 3 weeks AL culture.



## 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 them 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 for a few weeks at airlift culture (AL) thereby providing sufficient time to study pigmentation or de-pigmentation. It also allows to investigate skin differentiation and barrier formation over a substantial period of time.

