In vitro reconstructed model of human epidermis containing melanocytes for studying skin pigmentation and lightening

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Introduction

Products that modulate natural skin pigmentation are used to protect one's skin from UV irradiation (by increasing melanin content of the skin), or to treat skin pigmentation disorders, such as melasma, dark spots, solar lentigo, and other hyperpigmentation lesions. Clinical studies of such products are lengthy and often run for more than 3 months. We have developed the 3D reconstructed tissue culture model of human skin, which contains normal human melanocytes (NHM). Epidermal tissues can contain NHM of varying skin phototypes which follow the pigmentation level of the donor tissue, i.e., Black, Asian, or Caucasian. We have utilized this model to develop the protocol for studying skin whitening products *in vitro*. The tissues were treated topically three times a week over a two-to-three-week period to mimic consumer application. Several over-the-counter skin lightening products were evaluated in cultures containing NHM from Black and Asian donors. Over the treatment period, negative control cultures became increasingly pigmented with retention of normal epithelial morphology. In contrast, tissues treated topically with cosmetic skin lightening agents containing tyrosinase inhibitors such as kojic acid and magnesium ascorbyl phosphate remained lighter than the control cultures. The skin lightening effect on treated tissues was quantitatively evaluated for melanin content using a Solvable melanin assay and for skin brightness (L* value) using a hand-held spectrometer. Treated tissues showed significant changes in overall melanin content and brightness compared to control tissues. These results suggest that this model can provide valuable *in vitro* data for screening raw materials prior to the commencement of costly clinical trials and that it will be useful to study melanogenesis, skin lightening, and other pigmentation phenomena of the skin.

Methods and Results

Cell Sources: Normal human epidermal keratinocytes (NHEK) were isolated from neonatal foreskins or obtained from commercial sources. Normal human melanocytes were isolated from neonatal foreskins. Melanocytes were harvested from normal black (NHM-B), Caucasian (NHM-C), or Asian (NHM-A) skin tissue and are tested for infectious agents such as HIV-1, Hepatitis B, and Hepatitis C. Additional information on the MelanoDerm™ and EpiDerm™ models can be found in data and technical specification sheets available from MatTek Corporation.

Production of 3-D Organotypic Tissues: Primary NHEK and NHM were seeded onto microporous membrane inserts and cultured at the air-liquid interface for up to 21 days to produce the differentiated 3D MelanoDerm™ tissue model.

Melanin Assay: A quantitative assay to determine melanin content in the tissue was performed using the SOLVABLE™ melanin assay. Briefly, MelanoDerm™ tissues were placed in SOLVABLE™ at 60ºC overnight. The following day, samples were cooled and centrifuged to pellet any insoluble material. 200µl of each sample were added to a 96-well plate and read at 490nM. Synthetic melanin (Sigma) was carried through the above procedure and a standard curve was constructed so that melanin content of unknown samples could be determined.

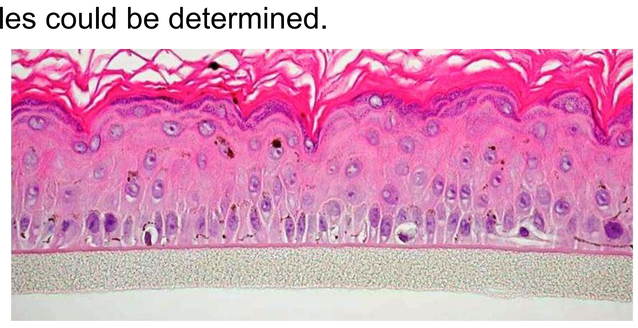

Figure 1: H&E Stained paraffin section of MEL-300-B cultured for 14 days showing melanin granules (400X).

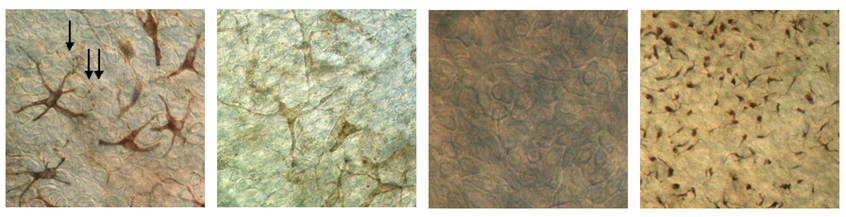

Figure 2: Top view of melanocytes in MelanoDerm™ (MEL-300) containing: **A)** NHM-B at Day 7 (magnification = 360 X) – arrows show melanin granules transferred to adjacent keratinocytes, **B)** NHM-A at Day 7 (360X), **C)** NHM-C at Day 16 (360 X), and **D)** NHM-C at Day 16, 24 hours after addition of DOPA (90X).

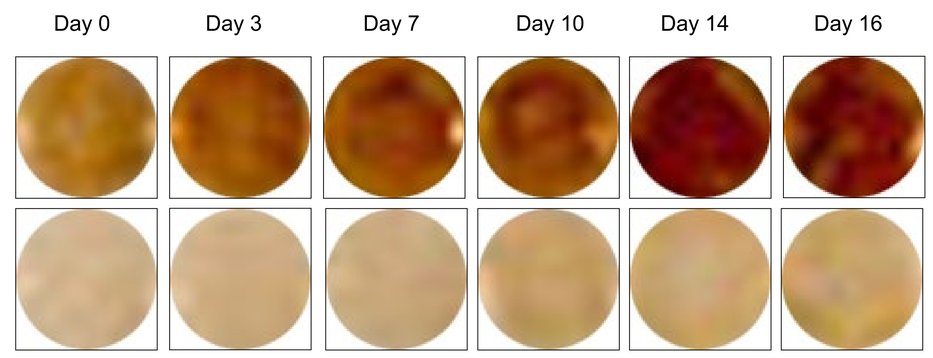

Figure 3: Comparison of progressive macroscopic darkening in MelanoDerm™ tissue (MEL-300) containing melanocytes harvested from black (MEL-300-B) or Caucasian donors (MEL-300-C)

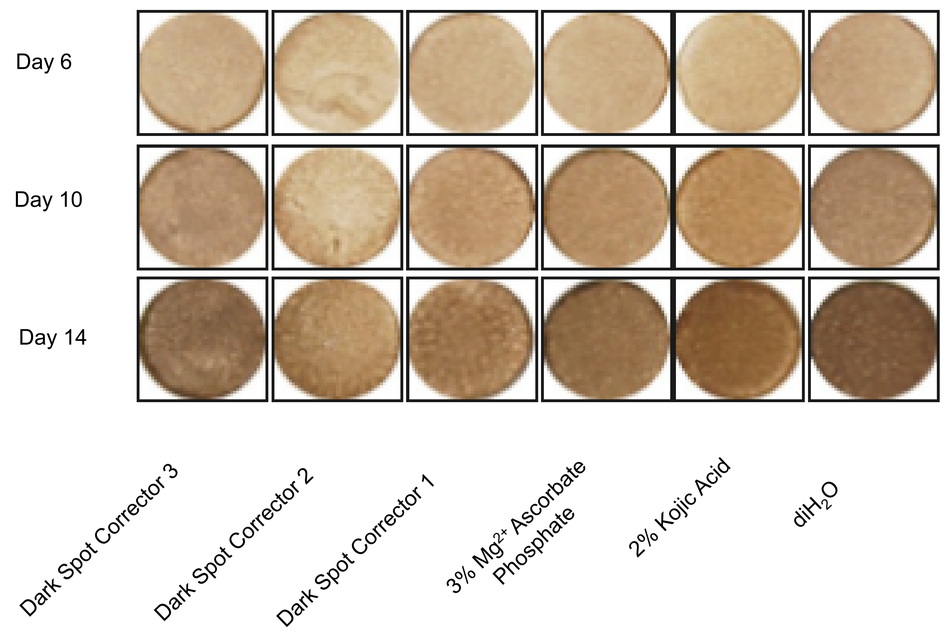

Figure 4: Effect of cosmetic ingredients and formulations on macroscopic darkening of MEL-300-B tissues.

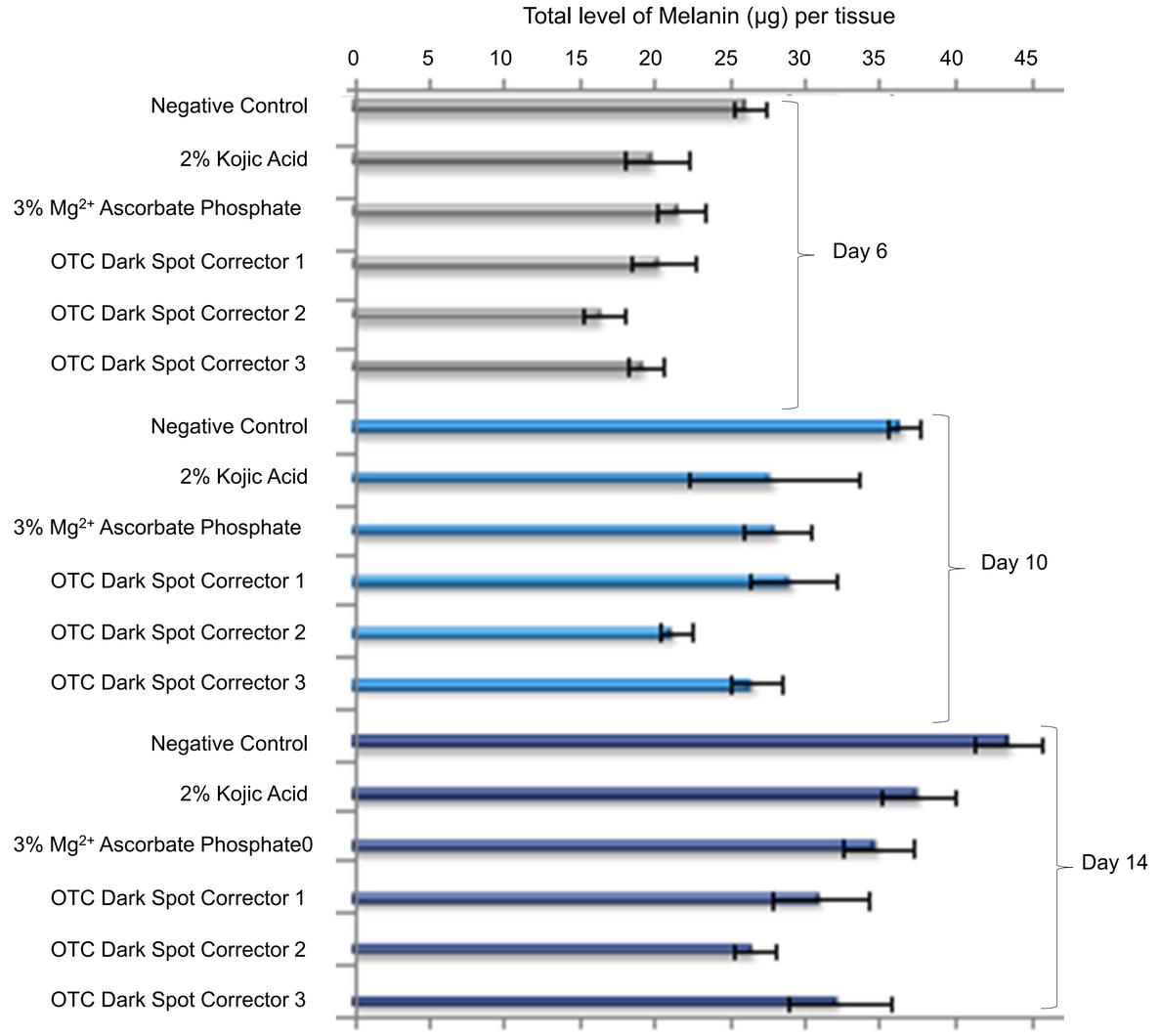

Figure 5: Effect of cosmetic ingredients and formulations on melanin production in the MelanoDerm™ (MEL-300-B) tissue model. The SOLVABLE™ melanin assay was used to quantify melanin levels in MEL-300-B tissues after 6, 10 and 14 days of treatment.

Conclusions

- MelanoDerm™ tissues can be cultured for up to 3 weeks and normal epithelial morphology is maintained.
- Melanocytes are highly dendritic and are present in the basal cell layer of the MelanoDerm™ tissue.
- Depending on the source of the melanocytes, the MelanoDerm™ tissue will darken to varying degrees. MEL-300-B > MEL-300-A > MEL-300C
- Differences in phototypes in the resulting tissue can be detected visually and with a quantitative melanin assay.
- The SOLVABLE melanin assay can be used to quantitate levels of pigmentation.
- Assessment of MelanoDerm™ skin lightening following treatment with topically or systemically applied cosmetic ingredients or formulations can be used in efficacy and claims substantiation studies.