Transferring pigmentation from forearm to face

Remo Campiche, Mathias Gempeler, Dominik Imfeld – DSM Nutritional Products
Sarah Gougeon, Marie Cherel, Magalie Roche – Newtone Technologies, Lyon, France

ABSTRACT

There is interest, particularly within the cosmetics industry, in understanding what pigmentation reactions and their modulation look like on the face. We have therefore developed methods that enable us to transfer pigmentation digitally from volunteers' inner forearms to their faces. Using these methods, we can predict facial pigmentation reactions, based on the reaction on surrogate body sites such as the forearm, without irradiating the face.

Irradiating the skin is an established method for studying pigmentation, UV damage and photoageing, and for inducing erythema to assess the efficacy and determine the sun protection factor (SPF) of UV filters. In order to develop and test active ingredients that can help prevent photoageing and modulate visible pigmentation, the cosmetics industry is particularly interested in studying photoageing and cutaneous tanning reactions.

Cosmetic scientists use skin irradiation to help them understand how to prevent pigmentation reactions, which provide a model for ageing-dependent hyperpigmentation and uneven skin tone[1-3]. Historically, UV radiation (UVR) with a wavelength below 400 nm was used to irradiate skin[4-6]. More recently, high energy visible (HEV) or 'blue' light, which has a wavelength of 400–500 nm, has also been used to induce cutaneous pigmentation[7-9].

From an ethical viewpoint there are concerns regarding the irradiation of skin. Both UVA and UVB have been shown to induce DNA damage extensively, such as the formation of 8oxo-guanosin moieties for UVA and cyclobutyl pyrimidine dimer formation for UVB[10,11].

UVA and UVB contribute significantly to photoageing by inducing oxidative stress events[12], inflammation[13,14] and the up-regulation of enzymes responsible for extracellular matrix (ECM) degradation, particularly matrix-metalloproteases[15-17]. As such, UVR is still considered to be the main contributor to both cutaneous ageing and skin cancers such as squamous cell carcinoma[18], basal cell carcinoma[19] and melanoma[20]. In addition, HEV has been shown to contribute significantly to photoageing and cosmetically relevant changes in skin pigmentation[21].

While it usually only takes from a few seconds to a couple of minutes to induce a skin reaction with UVR irradiation due to its high energy, it takes up to an hour to induce visible signs of skin damage comparable to real life outdoor situations with HEV irradiation[9]. Facial skin is constantly exposed to environmental threats, such as solar irradiation, pollution and weather conditions[22]. It is therefore especially prone to photoageing, dryness and inflammatory conditions.

Many enzymes responsible for inflammation and ECM degradation are up-regulated in facial skin compared to photo-protected skin[23]. Moreover, the skin barrier has been found to be weaker in facial skin than in other body sites[24]. Therefore, facial skin needs special care and protection.

Taking all this into account, it seems logical that artificially irradiating skin on the face for the purposes of science or to develop commercial skin care products should be avoided and would be of ethical concern. Nevertheless, the cosmetics industry, in particular, is interested in the effects of solar irradiation on the face, and irradiation on other, surrogate body sites is considered second best.

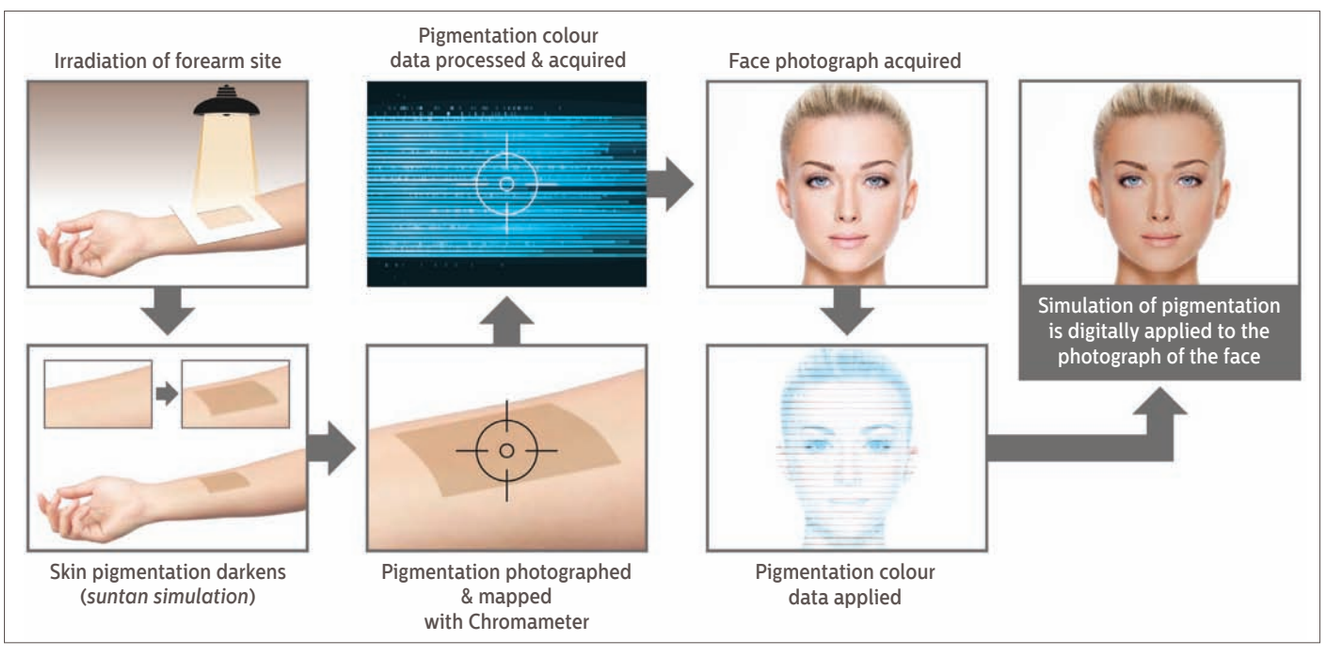

Figure 1: Flow chart illustrating method
Note: Pigmentation reaction is induced on the forearm, then digitally applied on face images to simulate facial tanning as if the face had been irradiated instead of the forearm

TABLE 1: FORMULATION USED IN METHOD 1

INCI	Product A (Placebo)	Product B (with microalgae)
Aqua	67.24	64.24
Sodium Gluconate	0.20	0.20
Propanediol	5.00	5.00
Xanthan Gum	0.20	0.20
Cetearyl Olivate; Sorbitan Olivate	4.00	4.00
Cetearyl Alcohol	1.50	1.50
Phenoxyethanol; Ethylhexylglycerin	1.00	1.00
Caprylic/capric Triglyceride	8.00	8.00
Octyldodecanol	10.00	10.00
Dimethicone	2.00	2.00
Hydroxyethyl Acrylate/sodium Acryloyldimethyl Taurate copolymer	0.40	0.40
Scenedesmus Rubescens Extract; Aqua; Phenoxyethanol	0.00	3.00
Citric Acid; Aqua	0.16	0.16
Parfum	0.30	0.30

TABLE 2: FORMULATION USED IN METHOD 2

INCI	% w/w
Aqua	62.98
Disodium EDTA, Aqua	0.05
Xanthan Gum	0.20
Propanediol	10.0
Ethoxydigl YCOL	2.00
Cetearyl Olivate, Sorbitan Olivate	3.00
Polysorbate 20	0.50
Stearic Acid, Palmitic Acid	1.00
Phytantriol	0.50
Isopropyl Myristate	4.00
Isostearyl Isostearate	4.00
Dicaprylyl Ether	4.00
Phenoxyethanol, Ethylhexylglycerin	1.00
Dimethicone	1.00
Paraffinum Liquidum	2.00
Hydroxyethyl, Acrylate/Sodium, Acryloyldimethyl Taurate Copolymer	0.50
SG-Peptide 1000ppm Solution	3.00
Sodium Hydroxide, Aqua	0.27

This background motivated us to develop methods for transferring visible pigmentation events on body areas like the forearms or back on one image to the face on another, or to simulate such events on the target image. The aim of the work presented here was to visualise and predict the impact of irradiation events on the face, even though these events were induced elsewhere on the body.

Reinhard *et al.* first described colour transfer between images in 2001[25]. Since then, different groups have further developed this method to improve and more realistically transfer colour from one image to another[26-28]. Most recently, a method that takes into account emotions in colour transfer has been developed[29].

For our study, we developed and described methods for transferring images of solar light-induced visible pigmentation, modulated with skin care actives, on the forearms to images of the face of individuals. Our methods use chromameter measurements of skin colour, which are then applied to the images to adjust facial colour to forearm colour.

Material & methods: 1

Figure 1 illustrating the methods used in the first test. We used a base formulation (placebo) and an active formulation consisting of the base formulation plus 3% of a commercial product containing Pepha®-AGE, a dry 2.5% extract of the green freshwater microalga *Scenedesmus rubescens*. Table 1 shows the base and active formulation.

We recently developed a robust method for irradiating skin with blue light with a wavelength of around 450 nm. In brief, skin on the inner forearm is irradiated with 60 J/cm^{-2} blue light each day, equivalent to what can be obtained on a clear summer day in central Europe at around midday for about one hour[30], for four days in a row to reach a cumulative dose of 240 J/cm^{-2}.

Healthy volunteers (33 per group, female Caucasian, Asian and multi-ethnicity, aged 21-41 with skin phototype III and IV) applied a base formulation and a formulation containing 0.075% of the microalgal extract to the irradiation site. Pigmentation reaction was measured using a Chromameter® CR400 device from Konica.

Measurements were taken on Day 0 just before the first irradiation, on Day 3 just after the last, then on Days 4, 10 and 28. Images were taken with a Nikon D7000 camera in the presence of a 48-patch colour chart from Newtone Technologies. Skin colour was measured on the cheeks using a Chromameter CR400 and facial images were acquired using a Canfield Scientific Visia CR device, again with a 48-patch colour chart. All images were colour-calibrated to remove possible lighting variations.

To simulate the pigmentation reaction on the face, the colour variations measured on the forearm were digitally applied to facial images of the subject acquired with Visia CR system. As the face and the forearms are slightly different in colour, facial colour was adjusted to match the forearm colour using L*a*b* chromameter values. For this, the RGB image was converted to Lab space, the delta L*a*b* were applied to every pixel and the image was converted back to RGB space.

Next, the colour variations measured at each time point were applied to the facial images using the same algorithm. The resulting images illustrate the simulated, blue light irradiation-induced tan over time for a base formulation (placebo) and an active formulation.

Materials & methods: 2

Our second test compound consisted of the base formulation plus 3% of a commercial product containing 1,000 ppm Syn-Glow™ pentapeptide (INCI: Benzoyl Dipeptide-18 D-Phenylalanyl Arginyl D-Tryptophan Dipropylamide Mesylate). This is a known melanocortin receptor-1 agonist[31] and the expected enhanced skin pigmentation was known to be stimulated more effectively after mild UV irradiation on the skin.

For this reason, we tested skin tanning on the forearms of 29 female subjects (27 Caucasians, one Asian, one Arab) with an average age of 33.4 ± 5.5. An inclusion criterion for skin tone on the volar forearm was an ITA value of 28-55, corresponding to phototype II and higher.

The test formulation (Table 2) was applied on a defined section of approximately 4 x 5 cm on the forearm twice daily for 14 days. On the midpoint of the application area, the skin was UV-irradiated with a spot 1 cm in diameter four

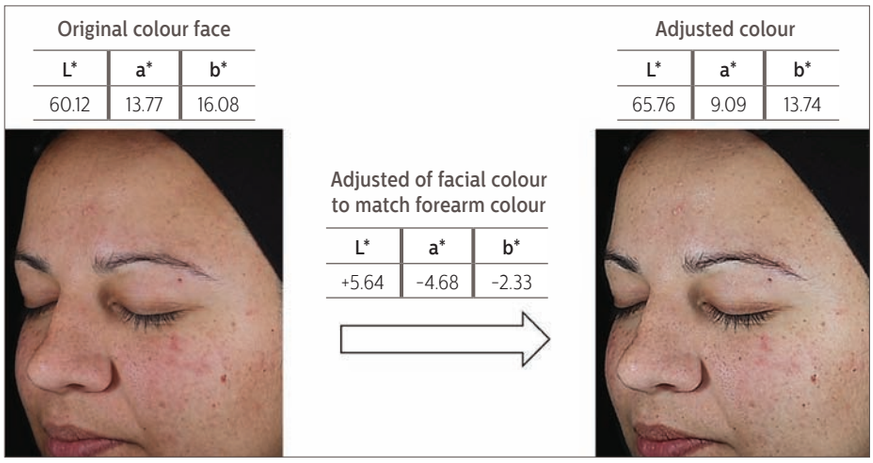

Figure 2: Adjusting face colour to match forearm colour in method 1

times in a row at 0.6 minimal erythemal dose (MED) from Day 1 to Day 4 to reach a total dose of 2.4 MED.

Chromameter measurements were taken at Days 1, 5, 10 and 15 at both the unirradiated and UV-irradiated skin sites. With this method it was possible both to measure the increase in skin tanning over the treatment time and to compare the treated skin to skin that had been UV-irradiated only.

Using a Solar Light300 W Single Port XPS 400 as the UV light source, irradiation was performed using a sun simulator which filtered and contained both UVA and UVB (UVA/UVB = 8.3571, using filters UG11 and WG320). The continuous emission spectrum ranged from 290 to 400 nm.

The system was used with a UV-transparent, flexible, light-fibre bundle to determine the MED and to irradiate the test areas at the required irradiation intensity on spots with a diameter of 1 cm each. The used intensity was 1.25 MED (irradiation on Day 2).

Six spots were needed to detect the MED. The UVB + UVA dose was increased from spot to spot by an increment of 25%. Then one irradiation at 0.6 MED per test area was performed daily for four days. The total dose received was then 2.4 MED.

To simulate the pigmentation reaction on an anonymous and aesthetic face, the colour variations measured by the Chromameter in terms of L*a*b* values were also applied to an average facial image computed from facial images acquired with Newtone's ColorFace system[32].

Specific morphological points were automatically detected on each image and all the images were registered toward the average position of each morphological point[32]. Then, the average facial image was computed by averaging all the images after registration.

The same algorithm was applied to average facial images, to visualise both average and individual colour variations. The resulting images illustrate the simulated, irradiation-induced tan over time for a base formulation (placebo) and an active formulation. Additionally, it was possible to simulate both the active and placebo colour variations on each hemi-face of the average facial images to highlight the difference between the two formulations.

Results: Method 1

Colour measurements on the cheeks of volunteer #3 yielded an L* of 60.12, an a* of 13.77 and a b* of 16.08. On the inner forearm, they yielded an L* of 65.76, an a* of 9.09 and a b* of 13.74. The colour measured on the cheek was therefore adjusted to the colour measured on the forearm.

This adjustment was applied to the image of the whole face (Figure 2). This resulted in a lighter skin colour, but still maintained any natural facial features such as colour heterogeneity or age spots. This approach was then applied to the same facial image but using the colour values of Days 3 (right after blue light irradiation) and 4 (one day after blue light irradiation).

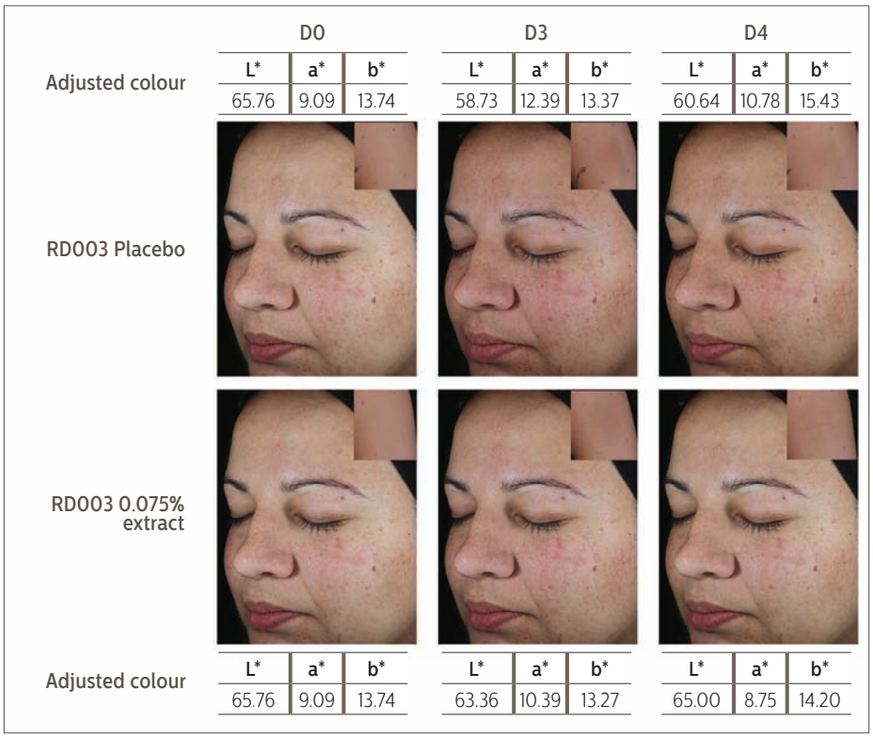

Figure 3: Adjusted face colour for several timepoints in method 1 showing volunteer #3
Note: Face colour was adjusted to Days 0, 3 & 4. Face visibly became darker and redder at D3 after blue light irradiation, this was prevented by the extract and colour intensity decreased again. Colours in these images represent the colours measured on the forearms (where irradiation took place) but are shown as projections on the face. Inserts show original images of the irradiated forearm area

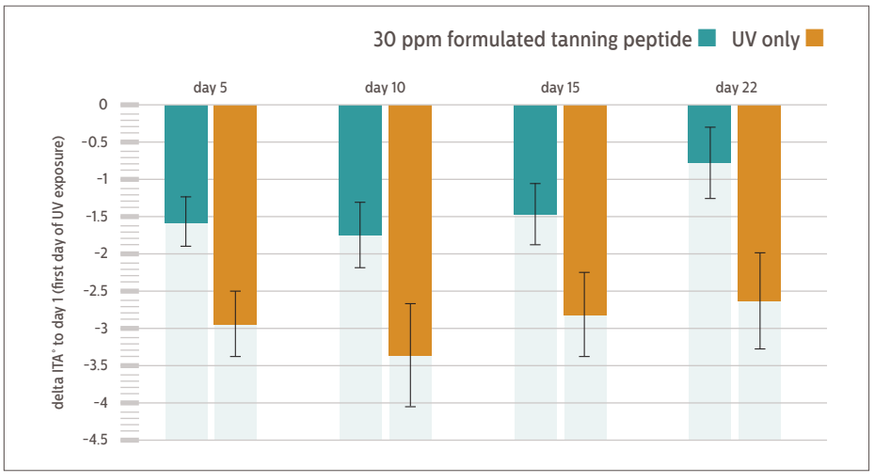

Figure 4: Chromameter measurements of UVR-irradiated sites on the forearm without peptide formulation (grey bars) & with 30 ppm SG-peptide formulation (blue bars) in method 2
Note: Delta ITA° values relative to first day of UVR exposure are shown. Error bars represent standard error of the mean. Significance with paired T-test of UV only compared to peptide treated. p values: D5 – 0.003, D10 – 0.029, D15 – 0.063, D22 – 0.009

In this way, we simulated the colour change induced by blue light on the forearm and the effect of the algae extract and the placebo on the face. It is evident that the algal extract protected the skin from blue-light-induced increases in skin pigmentation and skin reddening, while the placebo did not (Figure 3).

Results: Method 2

To obtain a clearly visible result after treatment with the SG-peptide, the skin was gently irradiated with sub-erythemal doses of UVR as described in the methods section. We expected the UV irradiation to stimulate the MC1-receptor expression, and as an outcome for the SG-peptide to induce pigmentation more effectively.

The changes in the delta ITA° values are shown in Figure 4 whereas the baseline ITA° values correspond to the level before the skin was UV irradiated for the first time[33]. As the graph shows, with gentle UV irradiation alone, changes in the ITA° values were very moderate.

Normally, a change within this level is not visible to the human eye. However, five days after treatment with the SG-peptide, the decrease in ITA° value, in other words the tanning effect, reached a statistically significant level (paired T-test, $p<0.01$, compared to UV only treated skin) that was also visible to the human eye.

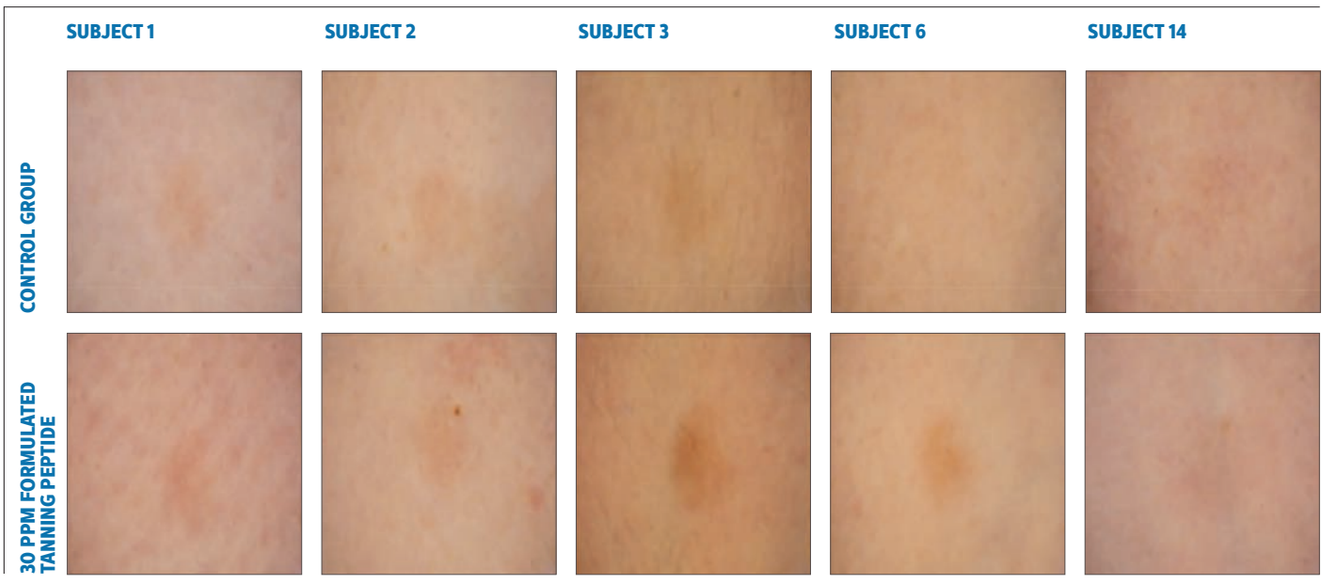

Figure 5: Irradiation sites on the forearm in method 2
Note: Both control group (upper panel) & active group (lower panel) were UV-irradiated. Control group is w/o SG-peptide. Images were taken on D15

Photographs of skin treated with the SG-peptide, taken on the forearm after 15 days, are shown (Figure 5) with darker tanned spots in the middle representing the UV-irradiated part of the skin. For a better visualisation of this tanning effect, we then transferred the data, as delta ITA° values, to a digitally created average facial image.

Instead of using the mean delta ITA° values from the complete study panel, for a more impressive visualisation we used the ITA° values from two selected subjects (#3 and 6) who showed a very positive individual tanning reaction (Figure 6a-b).

In the split face views, the left side shows the tanning level taken from forearm data after UV irradiation only, and the right side shows the tanning level taken from forearm data after UV irradiation and treatment with the SG-peptide. The prolonged tanning effect that can be seen seven days post treatment with SG-peptide (Day 22) is noticeable.

Discussion

In this paper, we describe two methods to digitally simulate tanning reactions on the forearm on the face. Irradiation with solar light, specifically in the UV or blue light range, causes readily visible changes in skin colour. In addition, it can induce erythema and is potentially harmful with respect to cellular damage, such as DNA mutations or creation of reactive oxygen species[34,35].

Because of this, irradiation experiments are often performed on surrogate body sites such as the back or the forearms, where the harmful effects still persist, but visible colour changes like pigmentation or reddening cannot readily be seen by others. Such irradiation may also cause a stinging or burning sensation, and this is likewise better accepted on areas such as the back or forearm instead of the face. Moreover, UVR is harmful to the eyes, which is another safety concern for facial irradiation.

Our methods provide a way to simulate facial solar irradiation, and the resulting visible pigmentation and colour changes, either on images of real faces or images of 3D artificial faces. Although they involve a mathematical transfer of colour, these methods give an idea of how colour changes induced on areas such as the forearm would look on the face without irradiating the face.

This method offers the advantage of simulating such pigmentation reactions on the whole face, whereas irradiation experiments are normally only performed on small areas and would therefore not show changes on a larger area. In addition, only small test areas are necessary on surrogate body sites. This makes it possible to test several different conditions per subject, making studies more time- and cost-efficient.

One limitation of these methods is that they show uniform colour changes over the whole face based on measurements on a single spot, which may not accurately represent real world colour changes. In fact, we have shown in other studies that changes in skin tone in Asian volunteers are different on different areas of the face[32]. This is also true for other facial skin parameters such as hydration and water content[36,37].

Spatial differences in facial colour change may be due to different facial characteristics such as thickness, sebum content or the distribution of melanocytes. In addition, tanning in forearm skin is not necessarily the same as in facial skin, due to differences in skin thickness or initial photodamage.

Along those lines, light of specific wavelengths may not have the same impact on forearm and on face due to for example non-photoexposed vs. photoexposed skin. As far as irradiation is concerned, differences may also be due to the three-dimensional topography of the face which leads to variation in irradiation intensity due to a steeper or flatter irradiation angle.

Our first method partially resolves this limitation, because we take the calculated colour of every pixel of the facial image as a starting point. This takes account of all differences in skin colour over the whole face, at least at the beginning (Figure 2). Only afterwards, when adjusting to forearm colour and calculating delta values between treatments and timepoints, is the colour shifted as a whole (Figures 2 & 3).

In method 2, we transfer forearm colour to a 3D-image of a simulation of a typical face which is composed of several individual face images. Average facial images are computed from several images, which are registered and averaged[32]. Here, any irregularities on the face disappear and the colour looks more even (Figure 6a-b).

The more even look and the homogenous skin tone achieved with method 2 is very useful for visualising treatment effects as they enable slight differences in colour variation to be seen more easily, whereas method 1 preserves the more natural and individual look of the skin's colour.

Another important advantage of both methods is the possibility for better standardisation of test conditions. As the tanning effect of blue light and the SG-peptide are also triggered by sunlight, the forearm skin could easily be protected using a long-sleeved shirt. However, such standardised light conditions would not have been applicable in a direct facial study.

In summary, we provide here two new methods with which we can simulate pigmentation reactions, induced by solar light irradiation, on the forearms.

Acknowledgements

We are grateful to the volunteers for their participation in the clinical studies. In addition, we would like to thank our colleagues at DSM Nutritional Products and Newtone Technologies for productive discussions throughout this project

PC

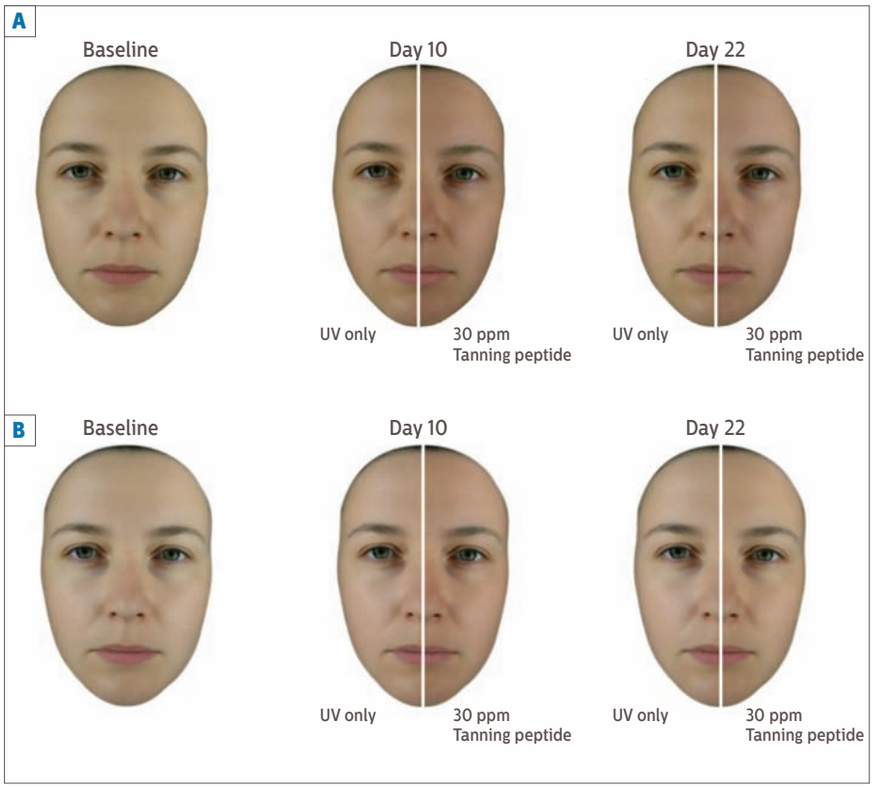

Figure 6: Transfer of tanning effect on forearm
Note: As seen in Figures 4 and 5, to average facial images in method 2. D10 & D22 show split face simulations of tanning UV-irradiation only (left side), and with UV-irradiation plus 30 ppm SG-peptide formulation (right side). a) Subject 3, b) Subject 6

References

1 Bissett, DL *et al.* Topical niacinamide reduces yellowing, wrinkling, red blotchiness & hyperpigmented spots in ageing facial skin. *Int. J. Cosmet. Sci.* 2004. 26(5):231-8
2 Sanches Silveira JE, Myaki Pedroso DM. UV light & skin aging. *Rev. Environ. Health.* 2014. 29(3): 243-54
3 Castanet J, Ortonne JP. Pigmentary changes in aged & photoaged skin. *Arch. Dermatol.* 1997. 133(10):1296-9
4 Marionnet C *et al.* UVA1-induced skin darkening is associated with molecular changes even in highly pigmented skin individuals. *J. Invest. Dermatol.* 2017. 137(5): 1184-1187.
5 Schallreuter KU *et al.* Regulation of melanogenesis: Controversies & new concepts. *Exp. Dermatol.* 2008. 17(5): 395-404
6 Cui R *et al.* Central role of p53 in the suntan response & pathologic hyperpigmentation. Cell. 2007. 128(5): 853-64
7 Duteil L *et al.* Differences in visible light-induced pigmentation according to wavelengths: A clinical & histological study in comparison with UVB exposure. *Pigment Cell Melanoma Res.* 2014. 27(5): p. 822-6
8 Duteil L *et al.* A method to assess the protective efficacy of sunscreens against visible light-induced pigmentation. Photodermatol. *Photoimmunol. Photomed.* 2017. 33(5): 260-266
9 Randhawa M *et al.* Visible light induces melanogenesis in human skin through a photoadaptive response. *PLoS* One. 2015. 10(6): e0130949
10 Chen H, Weng Q-Y, Fisher DE. UV signalling pathways within the skin. *J. Invest. Dermatol.* 2014. 134(8): p. 2080-5
11 Ichihashi M. *et al.* UV-induced skin damage. *Toxicology.* 2003. 189(1-2): 21-39
12 Rinnerthaler M *et al.* Oxidative stress in aging human skin. *Biomolecules.* 2015. 5(2): 545-89
13 Bald T *et al.* Ultraviolet-radiation-induced inflammation promotes angiotropism & metastasis in melanoma. *Nature.* 2014. 507(7490): p. 109-13
14 Saito P *et al.* The lipid mediator resolvin D1 reduces the skin inflammation & oxidative stress induced by UV irradiation in hairless mice. *Front. Pharmacol.* 2018. 9: 1242
15 Scharffetter K *et al.* UVA irradiation induces collagenase in human dermal fibroblasts *in vitro* & *in vivo*. *Arch. Dermatol. Res.* 1991. 283(8): 506-11
16 Herrmann G *et al.* UVA irradiation stimulates the synthesis of various matrix-metalloproteinases in cultured human fibroblasts. *Exp. Dermatol.* 1993. 2: 92-97
17 Watson RE *et al.* Damage to skin extracellular matrix induced by UV exposure. *Antioxid. Redox. Signal.* 2014. 21(7): 1063-77
18 Brash DE *et al.* A role for sunlight in skin cancer: UV-induced p53 mutations in squamous cell carcinoma. *Proc. Natl. Acad. Sci. USA.* 1991. 88(22): 10124-8
19 Schmitt J *et al.* Occupational UV exposure is a major risk factor for basal cell carcinoma: Results of the population-based case-control study FB-181. *J. Occup. Environ. Med.* 2018. 60(1): 36-43
20 Abdel-Malek ZA *et al.* Melanoma prevention strategy based on using tetrapeptide alpha-MSH analogs that protect human melanocytes from UV-induced DNA damage & cytotoxicity. *FASEB J.* 2006. 20(9): 1561-3
21 Sklar LR *et al.* Effects of ultraviolet radiation, visible light & infrared radiation on erythema & pigmentation: A review. *Photochem. Photobiol. Sci.* 2013. 12(1): 54-64
22 Krutmann J *et al.* The skin aging exposome. *J. Dermatol. Sci.* 2017. 85(3): 152-161
23 Raj N *et al.* Variation in the activities of late stage filaggrin processing enzymes, calpain-1 & bleomycin hydrolase, together with pyrrolidone carboxylic acid levels, corneocyte phenotypes & plasmin activities in non-sun exposed & sun-exposed facial stratum corneum of different ethnicities. *Int. J. Cosmet. Sci.* 2016. 38(6): 576-575.
24 Ya-Xian Z, Suetake T, Tagami H. Number of cell layers of the stratum corneum in normal skin - relationship to the anatomical location on the body, age, sex & physical parameters. *Arch. Dermatol. Res.* 1999. 291(10): p. 555-9.
25 Reinhard E *et al.* Colour transfer between images. *IEEE Computer Graphics & Applications.* 2001. 21(5): 34-41.
26 Wu F *et al.* Content-based colour transfer. *Computer Graphics Forum.* 2013. 32(1):190-203.
27 Yoo J.-D *et al.* Local colour transfer between images using dominant colors. *Journal of Electronic Imaging.* 2013. 22(3): 033003
28 Khan A *et al.* Fast colour transfer from multiple images. arXiv:1612.08927v1 cs.CV, 2016
29 Liu S, Pei M. Texture-aware emotional colour transfer between images. *IEEE Access.* 2018. 6: 31375-31386.
30 Campiche R *et al.* Pigmentation effects of blue light irradiation on skin & how to protect against them. *Int. J. Cosmet. Sci.* 2020. 42: 399-406
31 Jackson *et al.* Discovery of a highly selective MC1R agonist pentapeptide to be used as a skin pigmentation enhancer & with potential anti-ageing properties. *Int. J. Mol. Sci.* 2019. 20(24): 6143
32 Seroul P. *et al.* An image-based mapping of significance & relevance of facial skin colour changes of females living in Thailand. *Int. J. Cosmet. Sci.* 2020. 42(1): 99-107
33 Chardon A, Cretois I, Hourseau C. Skin colour typology & suntanning pathways. *Int. J. Cosmet. Sci.* 1991. 13(4): 191-208
34 Dupont E, Gomez J, Bilodeau D. Beyond UV radiation: A skin under challenge. *Int. J. Cosmet. Sci.* 2013. 35: 224-232
35 D'Orazio J *et al.* UV radiation & the skin. Int. J. Mol. Sci. 2013. 14(6): 12222-48
36 Voegeli R *et al.* A novel continuous colour mapping approach for visualisation of facial skin hydration & transepidermal water loss for four ethnic groups. *Int. J. Cosmet. Sci.* 2015. 37(6): 595-605
37 Egawa M *et al.* Visualisation of water distribution in the facial epidermal layers of skin using high-sensitivity near-infrared imaging. *Appl. Spectrosc.* 2015. 69(4): 481-7