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Quantification of skin sensitivity to ultraviolet radiation using ultra-wideband optoacoustic mesoscopy

Dear Editor,

Phototesting is used to assess individual sensitivity to ultraviolet (UV) radiation in order to determine adequate UV dosage for phototherapy[1]. In the standard procedure, small skin areas are exposed to increasing doses of UV radiation. The lowest UV dose that induces a delineated erythema at 24±2 h after UV exposure defines the minimal erythema dose (MED)[2]. Visual assessment is the gold standard for MED determination; however, it is prone to observer variability[3]. Optical methods have been considered to quantify the magnitude of erythema response. However, they are limited by light scattering therefore high-resolution is restricted to depths of <200 µm resulting in unreliable measurements[4,5].

Ideally, a quantitative method should offer a comprehensive observation of the skin and its microvascular structure, the latter exhibiting considerable inter- and intra-individual variations likely influencing the visual appearance of erythema formation[6]. In addition, it would ensure that precisely the same skin region of interest (ROI) is measured before and after UV-induced erythema development. Also, it should disentangle the effect of the melanin layer from the effect of the hemoglobin.

Optoacoustic techniques enable high-resolution imaging deeper than purely optical methods by resolving optical contrast at ultrasonic resolutions. They work by illuminating the ROI with short

laser pulses, stimulating the tissue to emit acoustic waves, which are detected by an ultrasound detector. Mathematical processing of the detected waves yields three-dimensional imaging of light-absorbing structures, such as blood vessels. Ultra-wideband Raster Scan Optoacoustic Mesoscopy (UWB-RSOM) in particular, is an optoacoustic imaging modality that, in the visible, allows visualization of skin structures and dermal microvasculature at depths of up to 1.5mm, reaching resolutions up to 7µm (axial) and 30µm (lateral)[7,8]. This performance allows detection and quantification of microvascular features typical of inflammatory skin diseases, such as psoriasis and eczema, inaccessible with other methods. We wanted to investigate whether UWB-RSOM could provide high-resolution, objective assessments of standard phototesting through comprehensive analysis of shallow and deep microvasculature responses.

Seven healthy volunteers (1 woman, 5 men; age range, 27-66 yrs; Fitzpatrick skin types II-III) participated in the study, one of whom was used as non-irradiated control. Six skin areas measuring 2.8 x 2.5. cm in each of six participants were exposed to increasing doses of UVB-rich radiation (ultraviolet light type B; wavelength 275-365 nm). The control participant did not receive UV light. After 24±2 h an experienced clinician determined the MED of each volunteer based on visual assessment. Before UV irradiation and at the time of clinical evaluation the same sections of dermal microvasculature were assessed with UWB-RSOM by means of a protocol based on ink fiducial markers (see Fig. 1A-B). UV-induced changes in dermal total blood volume (dermal blood fraction) were quantified from identical parts of the dermal microvasculature. The ROIs were defined using microvascular bifurcations as reference from the en-face cross-sectional images. Six non-irradiated skin areas in the control participant were imaged and quantified likewise at 0 and 24 h.

UWB-RSOM cross sectional views reveal the effect of the erythematous skin reaction on the whole microvascular structure after exposure to the MED (Fig. 1A). As expected, the effect is more pronounced for higher doses (Fig. 1B). UWB-RSOM shows UV-induced recruitment of vessels that were not previously perfused. The images also reveal vasodilation, visible as an increase in vessel diameter at different depths. Smaller micro-vessels and capillaries, which emit higher-frequency ultrasound signals, are shown in green; larger micro-vessels emitting lower-frequency signals are shown in red.

Fig. 1C shows that the blood fraction, as measured by UWB-RSOM, increased approximately linearly as a function of UV dose: The UV dose of 25 mJ/cm^2 below the visual MED triggered an average increase in blood fraction of 5.1% (±5.3%) and the highest dose, an average increase of 49.6% (±25.4%). The control measurement showed a negligible average change in blood fraction of -1.6% (±5.6%). Individual vessel diameters show a similar trend (data not shown).

Our results demonstrate that UWB-RSOM allows direct monitoring and quantification of UV-induced erythema in phototesting with unprecedented spatial precision. We imaged and quantified the effect of increasing doses of UV light on identical microvascular regions through the entire depth of the skin, directly observing vasodilation and vessel recruitment as a function of macroscopic erythema. The results, moreover, indicate that UWB-RSOM could be a useful tool to detect the suberythemal response of the skin to UV radiation, which might increase the sensitivity of phototesting. The UWB-RSOM prototype has certain technical limitations including motion artefacts and slight variations in laser energy which may explain changes in blood volume in only five of six phototested skin areas exposed to the MED. Our findings, however, suggest that UWB-RSOM holds potential to improve the accuracy of phototesting.

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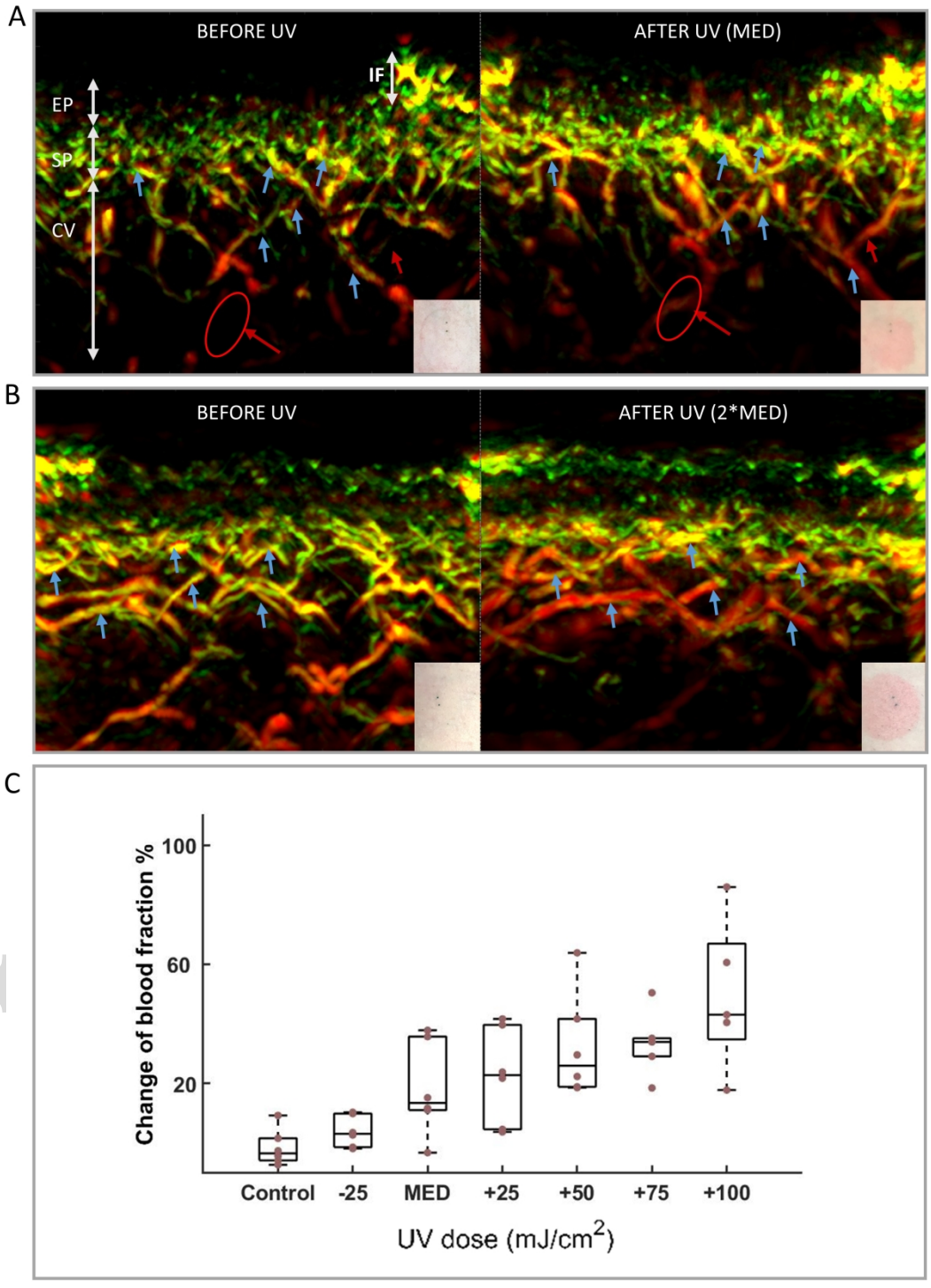

Figure Legend:

Figure 1: A. Same section of the dermal vasculature before and 24 hours after UV irradiation using the MED. Vasodilation (blue arrows) and vessel recruitment (red arrows) can be observed. SP: Sub-epidermal plexus. CV: Connecting vessels. IF: Ink fiducial marker. Insets: Clinical Pictures. **B.** Same as A but exposure to 2.5-fold MED. **C.** Change in blood fraction corresponding to all subjects irradiated with UV light and the control non-irradiated subject after 24 h. Doses are expressed relative to the individual MED.