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Assessment of advanced glycated end product accumulation in skin using auto fluorescence multispectral imaging

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ABSTRACT

Several studies have shown that advanced glycation end products (AGE) play a role in both the microvascular and macrovascular complications of diabetes and are closely linked to inflammation and atherosclerosis. AGEs accumulate in skin and can be detected using their auto fluorescence (AF).

A significant correlation exists between AGE AF and the levels of AGEs as obtained from skin biopsies. A commercial device, the AGE Reader, has become available to assess skin AF for clinical purposes but, while displaying promising results, it is limited to single-point measurements performed in contact to skin tissue. Furthermore, in vivo imaging of AGE accumulation is virtually unexplored.

We proposed a non-invasive, contact-less novel technique for quantifying fluorescent AGE deposits in skin tissue using a multispectral imaging camera setup (MSI) during ultraviolet (UV) exposure. Imaging involved applying a region-of-interest mask, avoiding specular reflections and a simple calibration. Results of a study conducted on 16 subjects with skin types ranging from fair to deeply pigmented skin, showed that AGE measured with MSI in forearm skin was significantly correlated with the AGE reference method (AGE Reader on forearm skin, $R=0.68$, $p=0.005$). AGE measured in facial skin was borderline significantly related to AGE Reader on forearm skin ($R=0.47$, $p=0.078$). These results support the use of the technique in devices for non-touch measurement of AGE content in either facial or forearm skin tissue over time.

1. Introduction

Advanced glycation endproducts (AGEs) are long-term indicators of metabolic and glycemic stress that can be found in human skin. They derive from the modifications of proteins or lipids that after contact with aldose sugars become glycated [1]. Factors that affects the formation of AGEs are: extended periods of hyperglycemia; oxidant stress in the cellular environment; and the rate of turnover of proteins for glycoxidation [2].

AGEs can also be absorbed through the diet [3]. Foods high in protein and fat are especially rich in AGEs. In addition, increased cooking temperatures, like broiling and frying, and increased cooking times lead to increased amounts of AGEs [4].

Inside the tissue, AGEs can alter cell structure and function, contribute to diabetes related micro- and macrovascular complications [5], and may modify the extracellular matrix [6]. They may also lead to the release of free radicals [6], block the activity of nitric oxide inside the endothelium [7] and increase the amount of reactive oxygen species [8]. When the AGEs have been formed inside the tissue, their turnover time is very long.

Considering the negative effects of AGEs in tissue, an objective way of quantify such an accumulation is of clinical value. Since several AGEs exhibit characteristic fluorescence, this physical effect can be exploited to perform a non-invasive assessment of accumulation increases into human tissues. Significant correlations have been found between skin autofluorescence (AF) and levels of skin AGEs like pentosidine, as obtained from skin biopsies when studying e.g. diabetes mellitus [9]. Skin AF can be used as a predictor for assessing how diseases with increased cardiovascular risk develops. Koetsier et al. [10] suggest that UV stimulations by a broad excitation range of 355–405 nm, is adequate for inducing AF for diagnostic purposes.

A commercial device, the AGE Reader (Diagnoptics Technologies B.V., Groningen, The Netherlands), has become available to assess skin auto fluorescence for clinical purposes [11]. A black light tube, with a peak wavelength of 370 nm is used to illuminate a small region of the skin on the forearm. An optical fiber detects the emission and reflected excitation light, and a spectrometer is

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used to measure the intensity spectrum. This device is limited to single-point measurements performed in contact to forearm skin.

The aim of this work is to demonstrate the feasibility of quantifying fluorescent AGE deposits in skin tissue using a two-camera setup with optical band pass filters during ultraviolet (UV) exposure. Such a tool enables the analysis of the spatial distribution of AGE in tissue and can be used in devices devoted to remote monitoring and self-monitoring whenever trends in AGE tissue content are required to be traced over time, such as a smart mirror. Incorporating physiological measurements in a smart mirror will increase its medical value [12]. As a proof-of-concept, the AGE level quantified with the proposed camera setup will be compared to readings from a commercial instrument for single point measurements performed in contact to arm skin tissue.

2. Material and methods

2.1. AGE auto fluorescence and quantification

The possibility for detecting the AGE related auto fluorescence spectrum in an imaging setup was initially evaluated in a pilot study using a HSI (Hyper Spectral Imaging) system consisting of a monochromatic camera (Dolphin F-145B, Allied Vision Technologies GmbH, Germany) with an attached tuneable LCTF (Liquid Chrystal Tuneable Filter; VariSpec VIS 7 nm FWHM, PerkinElmer Inc., US) band-pass filter. This setup was capable of capturing HSI data in the 445–720 nm range. The initial evaluation encompassed HSI data taken from the volar side of the forearm during UV exposure (365 nm; R130-365-N, Smart Vision Lights, US) using LED light below 400 nm (Fig. 1). In total, 18 subjects were evaluated. This data display a consistent spectral appearance similar to that of AGE auto fluorescence [13,14], with a peak located at 480–490 nm (Fig. 2). This result indicates that an MSI camera setup capable of capturing the light above 450 nm would be suitable for detecting UV induced AGE auto fluorescence. In order to minimize the influence from the strong blood-related absorption peaks above 500 nm, the detection of auto fluorescence should be limited to the 450–500 nm spectral band.

The amount of backscattered auto fluorescence depends not only on the amount of fluorophores in tissue but also on the intensity of the excitation UV light. A simple, yet effective way of quantifying the amount of excitation light is to detect the diffusely backscattered UV light. As proposed by Graaff et al. [13], the excitation intensity dependency can be canceled out by calculating the normalized auto-fluorescence ratio

$$AF=\frac{I_{em}}{I_{ex}}, \quad (1)$$

where I_{em} is the mean detected auto fluorescence (450–500 nm) and I_{ex} is the mean detected diffusely backscattered UV light. Using a 365 nm UV LED light source, I_{ex} can be quantified by detecting light below 400 nm.

2.2. MSI camera setup and image acquisition

The multispectral imaging camera setup (Fig. 3) consisted of two monochromatic cameras (FL3-U3-32S2M-CS, Point Grey Research Inc., CA) placed closely beside each other, about 35 mm apart (center–center separation), with optical band-pass filters mounted in front of a 8 mm C-mount lens (M118FM08, Tamron Co. Ltd., Japan). The optical filters where selected specifically to pinpoint both the amount of diffusively backscattered UV light (310–390 nm FWHM, U-360 UV, Edmund Optics Inc, US), I_{ex}, and the

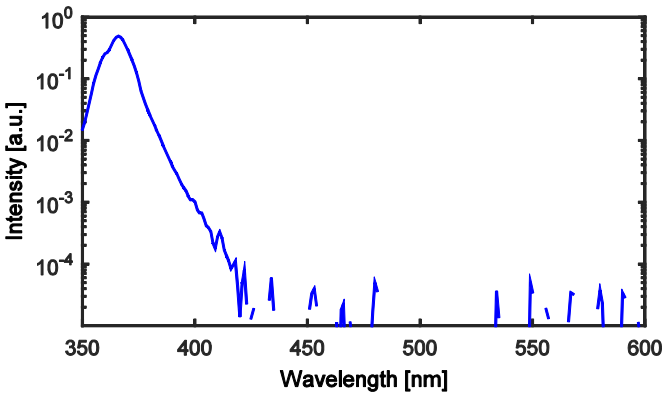

Fig. 1. Emission spectra of the 365 nm UV LED excitation light source.

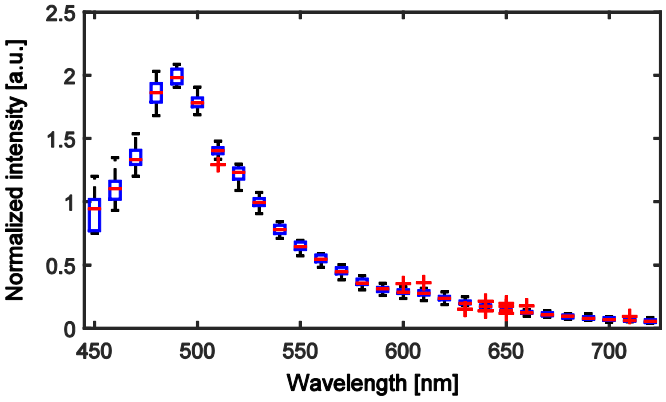

Fig. 2. Box-plot of 18 auto fluorescence spectra, each normalized by their average intensity. The spectra were captured from the volar side of the forearm when exposed to 365 nm UV LED light. An auto fluorescence peak is shown at 480–490 nm.

Fig. 3. Two-camera MSI system with custom optical filters.

amount of AGE related auto-fluorescence (450–500 nm, 6 OD outside the band pass region, Edmund Optics Inc, US), I_{em}. UV exposure of the tissue was performed using an 8 W 365 nm LED ring light (R130-365-N, Smart Vision Lights, US).

Image acquisition was carried out using a software sync scheme allowing for simultaneous acquisition of images from both cameras. For each camera a set of 10 images where recorded with the UV LED turned both off and on. The switching of the light settings was computer controlled, allowing for the acquisition off all images to be completed in less than 2 s. This rapid acquisition scheme allowed for only minor movement artifacts due to subject head or arm movement. The sets of 10 images where averaged in

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