Abstract: In the present study, the distribution of the carotenoids as a marker for the complete antioxidative potential in human skin was investigated before and after the topical application of carotenoids by in vivo Raman spectroscopy with an excitation wavelength of 785 nm. The carotenoid profile was assessed after a short term topical application in 4 healthy volunteers. In the untreated skin, the highest concentration of natural carotenoids was detected in different layers of the stratum corneum (SC) close to the skin surface. After topical application of carotenoids, an increase in the antioxidative potential in the skin could be observed. Topically applied carotenoids penetrate deep into the epidermis down to approximately 24 μm. This study supports the hypothesis that antioxidative substances are secreted via eccrine sweat glands and/or sebaceous glands to the skin surface. Subsequently they penetrate into the different layers of the SC.

In vivo Raman spectroscopy detects increased epidermal antioxidative potential with topically applied carotenoids

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1. Introduction

The skin is a specific barrier of our organism to the environment. It can be easily investigated by non-invasive optical methods [1–5]. UV irradiation and hazardous environmental substances induce the production of free radicals in the skin [6–8]. These reactive molecules have negative effects on cells and cell compounds [9]. The formation of free radicals in the skin by natural light is the main reason for skin aging. Free radicals destroy the collagen elastin fibers. As a result, furrows and wrinkles appear [10].

The human organism has developed protection strategies against the destructive action of the radicals in form of an antioxidative potential [11–13]. Typical antioxidant substances in the skin are: vitamins A, C and D, enzymes and carotenoids [13,14]. Carotenoids are one of the most important antioxidants in the human skin. 70% of the carotenoids are beta-carotene and lycopene [11].
Darvin et al. [10], presented a method for the non-invasive \textit{in vivo} determination of carotenoids in human skin [15,16]. It was shown that individuals with high levels of carotenoids in the skin have less furrows and wrinkles than individuals with lower antioxidant levels. Most of the antioxidant substances, including the carotenoids, cannot be produced by the human organism itself. Therefore, they have to be incorporated by nutrition rich in fruit and vegetables or by antioxidant containing nutraceuticals. Besides oral supplementation, the concentration of antioxidants in the skin can be increased by their topical application [17]. This is an essential strategy for anti-aging creams, in addition to the improvement of SC hydration. In the present study, the penetration of an anti-aging cream containing carotenoids into the epidermis of human skin was investigated \textit{in vivo} by Raman microscopy at different time points.

2. Materials and methods

2.1. Volunteers

The investigations were performed on 4 healthy volunteers (2 men, 2 women), aged between 32 and 50 years under standardized conditions. A written informed consent had been obtained from the healthy volunteers prior to the start of the study.

2.2. Topically applied substances

A commercially available anti-aging cream, kindly provided by Lancaster, was applied onto the forearm of the volunteers at a concentration of 1 mg/cm$^2$. The anti-aging cream contained 0.3% carotenoids.

Raman measurements were performed using a model 3510 Skin Composition Analyzer, dedicated for \textit{in vivo} skin measurements (River Diagnostics, Rotterdam, The Netherlands). The axial spatial resolution is 5 \textmu m, the laser excitation wavelength is 785 nm and the measurement stage includes confocal sampling optics. For the measurement, the volar forearm of the volunteer was placed on a fused silica window mounted in the measurement stage. Laser light was focused in the skin with a microscope objective located under the window. The location of the laser focus relative to the skin surface can be accurately and automatically varied. Raman fingerprint spectra (400 – 1800 cm$^{-1}$) were recorded from the skin surface down to a depth of 24 \textmu m, in 2 \textmu m steps. In this way, detailed Raman profiles were acquired across the stratum corneum. The measurement time for one spectrum was 10 seconds. Relative carotenoid concentrations were calculated from the Raman profiles, following the method described by Caspers et al. [18,19]. Briefly, a set of reference spectra of the major skin constituents is fitted to the Raman spectra of the arm. To correct for variations in the absolute Raman intensity, which decreases at greater distance to the skin surface, the fit coefficients were normalized on the Raman signal of keratin, which is the dominant dry mass fraction in the stratum corneum. The procedure resulted in the local contents of carotenoids in the stratum corneum, relative to the amount of keratin.

The carotenoid concentration in the skin was measured before and 30 min and 60 min after application of the anti-aging cream. The spectrum of carotenoids is depicted in Fig. 1. A typical spectrum of untreated skin (volar forearm of a healthy volunteer 10 \textmu m depth) is shown in Fig. 2.
3. Results

The carotenoid signal can be observed at 1521 cm$^{-1}$. Depth concentration profiles of natural carotenoids in the stratum corneum are presented in Fig. 3. The reproducibility of the measuring method is demonstrated by determining the carotenoid distribution in human skin in the same field at two slightly different locations (volar forearm). Similar distribution profiles were obtained (Fig. 3).

The carotenoid concentration clearly increased after the application of the carotenoid containing anti-aging cream. The typical distribution of the carotenoids in the SC at different time points after application and penetration is demonstrated in Fig. 4 for 2 volunteers. The highest concentration of natural carotenoids in untreated skin can be detected close to the skin surface (4–8 μm). The concentration appears to level off at a depth of approximately 15 μm (Fig. 3).

30 min after topical application of the anti-aging cream, a rather strong increase in carotenoid concentration at a depth of 4–8 μm can be observed. In addition, the depth distribution in the SC broadened up to 20 μm. After 60 min, the concentration of the carotenoids in the SC at a depth of 4–8 μm was reduced to the initial level detected at baseline. However, the carotenoid concentration in deeper parts of the SC remained elevated up to a penetration depth of approx. 24 μm. This means that the carotenoid distribution profile in the SC became broader and flatter.

4. Discussion

Antioxidant substances protect the human skin from the destructive action of free radicals, which are known to cause a number of skin damages, including skin aging [10]. Since most of the antioxidant substances are not produced by the human organism, they must be incorporated e.g., by nutrition [12]. Fruit and vegetables, in particular, contain high amounts of antioxidants. Darvin et al. demonstrated that different volunteers have different levels of carotenoids in the skin, depending on their lifestyle [20]. The carotenoids represent a marker for the whole antioxidative potential of the human organism due to their protective properties preventing each other from the destructive action of the free radicals [11]. In the present study, it was shown that the highest concentration of the carotenoids could be found close to the skin surface around the upper cell layers of SC, in the case of the untreated natural skin. Thiele et al. have shown that some antioxidants are delivered by sebaceous glands [13]. In the present measurements, the carotenoid concentration in the upper 1–3 μm of the SC was found lower than at a depth of 5 μm. This may be explained by the naturally occurring photo-chemical degradation of carotenoids by ambient UV light. Furthermore it has to be taken into account that the penetration depth of the UV photons is only a few micrometers in keratinic materials. As a result of the topical application of the anti-aging cream, the concentration of carotenoids in the upper part of the SC was strongly increased 30 min after application. A maximum could be detected at a depth of approx. 4–8 μm. Over time the antioxidants penetrated...
deeper into the stratum corneum, which had a thickness of approx. 24 μm at the body site under investigation. After 60 min, the carotenoids penetrated up to the boundary of the SC to the living epidermis (up to 24 μm). The distribution profiles of the carotenoids in the SC became broader and, subsequently, flatter. These penetration characteristics can be explained by the physiological properties of the antioxidants. Taking into consideration their natural distribution in the SC, where the highest concentration was detected close to the skin surface, it has to be expected that the carotenoids are delivered by sebum and sweat secretion. Reaching the skin surface, they penetrate into the skin like topically applied carotenoids. Such penetration characteristics were observed by Thiele et al. [14,21] for the antioxidant substance vitamin E. A transport of the carotenoids onto the skin surface by the continuous renewal of the SC is improbable, because in this case the concentration profile of the carotenoids should have an inverse tendency – the highest concentration should be on the boundary between the SC and the living epidermis and the lowest concentration on the skin surface.

If the carotenoids are soluble in the sweat, they should penetrate efficiently into the SC [22]. The deep penetration of the antioxidants into the SC has the advantage that the substances cannot easily be removed by washing or textile contact. This implies that the protection efficacy has a long-term character.

The present study demonstrates that Raman microscopy is a suitable method for the investigation of the distribution of the carotenoid antioxidant substances in human skin under in vivo conditions. This method allows to study penetration kinetics further over time. Taking into consideration that the carotenoids represent markers for the complete antioxidative potential, spectroscopic measurements are an efficient tool for the development and evaluation of anti-aging strategies.

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References