Correlation between Blood Flow and Various Physiological Parameters in Human Skin

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Abstract. Blood flow is an important parameter of the human organism and skin physiology. It correlates with skin temperature, penetration of active substances through the skin, delivery of antioxidants and development of disease symptoms, especially during inflammatory processes. Dr. Alexander Vasilyevich Priezzhev played a major role in the investigation of blood circulation, cell aggregation and disease correlation, as well as in the development of an aggregometer to determine the rheology of human blood flow. The following article provides an overview of the work performed at the Charité – Universitätsmedizin Berlin, Department of Dermatology and Allergology, Center of Experimental and Applied Cutaneous Physiology, partly in cooperation with Dr. Priezzhev, on the correlation of blood flow and various physiological parameters in human and animal skin *in vivo*. © 2022 Journal of Biomedical Photonics & Engineering.

Keywords: glucose; carotenoids; hemoglobin; capillaries; flowmeter; Raman spectroscopy; FLIM.

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1 Introduction

Blood flow serves as an important oxygen and nutrients delivery system and can be used for determining physiological parameters in the human organism and especially in the skin. Application of non-invasive methods is advantageous and currently popular in *in vivo* skin research. This concerns, but is not limited to the determination of the glucose concentration in blood samples using attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy [1, 2] as well as the analysis of the influence of topically applied substances on the blood flow using laser Doppler flowmetry [3–5]. Antioxidants like carotenoids beta-carotene and lycopene could be detected in the blood plasma correlating to the antioxidant status of human skin and reflecting health conditions in animals [6]. Non-invasive measurements of carotenoids in the skin are limited to the application of Raman spectroscopy and reflection spectroscopy [7–9]. The *in vivo* investigations of the cutaneous antioxidant status were carried out both on humans and in veterinary medicine on cattle [6, 10]. Furthermore, blood capillaries were visualized non-invasively in human skin using two-photon excited fluorescence lifetime imaging [11], which extends the application of confocal laser scanning microscopy and optical coherent tomography in dermatology [12–14].

Dr. Alexander Vasilyevich Priezzhev has been working in this field for years and achieved important biophysical results mainly focusing on the rheological properties of human red blood cells [11, 15–21]. The following article summarizes results in this area that have been achieved at the Center of Experimental and Applied Cutaneous Physiology in the past, partly in cooperation with Dr. Priezzhev.

2 Results and Discussion

2.1 Two-Wavelength CO_2 Laser for Determining the Glucose Concentration in Blood Samples

The determination of the glucose concentration in human blood is an important necessity in daily medical care. High blood sugar levels in diabetic patients can cause,

among others, vision problems, cardiovascular disease, kidney disease and pregnancy complications. Test strips are most commonly used to screen glucose levels. Accurate determination of blood glucose levels requires the use of more complicated systems. This includes, for instance, attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy. The sedimentation of the red blood cells, which contain a part of the blood glucose, on the ATR surface proves to be problematic with this method. Therefore, a two-wavelength-CO_2-laser was used for the following investigations. One wavelength λ_1 is at the maximum of the glucose absorption band in blood at 1080 cm^{-1}. The second wavelength λ_2 is set to a minimum absorption of glucose at 950 cm^{-1}. With this, the background can be measured. The ratio of both wavelengths is determined, which correlates well with the glucose concentration in whole blood samples. The strong linear correlation ($R^2 = 0.997$) has been obtained with a glucose concentration in the range of 40–400 mg/dl [1].

2.2 Influence of Topically Applied Active Substances on the Blood Flow

Topically applied drugs can affect the blood flow, i.e. especially the blood pressure, in the human organism. Such changes can be determined using a laser Doppler flowmeter. In the present case, the kinetics of vasodilation caused by the topical application of benzyl nicotine were investigated. This flowmeter measures the blood flow in the superficial dermal plexus and the deeper lying larger capillaries simultaneously and indirectly by determining the flow velocity [22].

Both sets of data were compared with the skin temperature and redness as a clinical sign of vasodilatation. The investigation was carried out with a flowmeter from the Laser- und Medizin-Technologie GmbH (LMTB) [22]. Here, the radiation of a laser diode at 785 nm was used. A skin area of 7 mm^2 was analyzed. No correlation of the blood flow with neither the skin temperature nor the redness was observed, which shows that these biological parameters are unsuitable for measuring the kinetics of the microcirculation after the topical application of drugs [23]. These results indicate the transport of the drug with the blood from the upper to the deeper capillaries. A linear proportionality between the blood flow of the superficial micro capillaries and the larger capillaries in deeper skin layers indicates that the drug is transported via the blood.

In addition, benzyl nicotinate could be used to investigate the difference between follicular penetration and intracellular penetration of topically applied drugs. So far, it has been difficult to differentiate between follicular penetration and intracellular penetration of topically applied substances. Spatially resolved measurement methods are required for investigations into follicular penetration. These measurements were possible only after the development of a method for the artificial closure of the hair follicles. So, it became possible to differentiate between these two penetration routes [24].

This study was performed on the hairy chest skin of men, with the hair carefully clipped with scissors. Afterwards the hair follicles were closed with a drop of a lacquer-wax mixture. In a second experiment with the same subjects at the same skin site, the follicles were left open and an equal number of drops of the lacquer-wax mixture were placed next to the follicles to occlude the same amount of skin surface. A comparison of the two penetrating amounts of the active ingredient provides information on the influence of follicular penetration compared to intracellular penetration.

In these investigations, the laser Doppler flowmeter developed by LMTB was used again. Two typical spectra are shown in Fig. 1. In the case of open follicles, the active substance can reach the blood circulation very quickly. An intense peak of the benzyl nicotinate concentration developed in short time. When the hair follicles are occluded, the drug must reach the blood flow system intracellularly through the lipid layers around the corneocytes [25]. This process took longer and was much more linear over time [4].

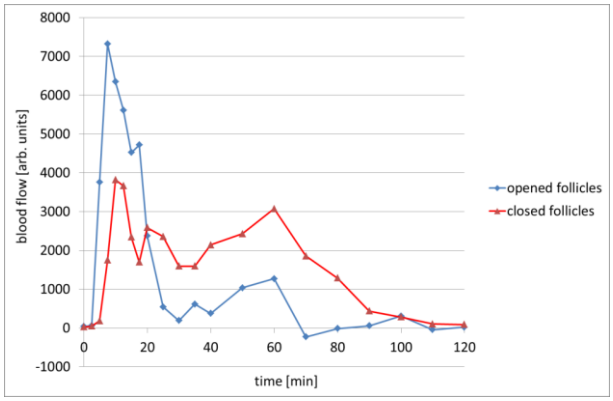

Fig. 1 *In vivo* changes of the blood flow after topical application of benzyl nicotinate on the skin of volunteers measured using laser Doppler flowmetry. The Fig. is adapted from Ref. [4].

2.3 Determination of Antioxidants in Blood Using Raman Spectroscopy

Different antioxidants are continuously circulating in the blood maintaining antioxidant defense of the tissue and organs. Carotenoids are fat-soluble molecules with a powerful antioxidant capacity against reactive oxygen species [26–28]. Carotenoids are dissolved in the blood plasma [29] and have a beneficial function in the human skin [6]. Carotenoids can be quantified separately using high performance liquid chromatography [9, 30] and as a total concentration using photometric absorption at 450 and 525 nm [31] or by Raman spectroscopy [29] directly in blood plasma samples. All these methods are widely used in practice. We have shown that multiple spatially resolved reflectance spectroscopy can be used to determine the total carotenoid concentration in the blood non-invasively measuring *in vivo* through the thenar skin

area of the palms [8]. A strong correlation (R = 0.79) between the directly and indirectly measured carotenoids in the blood was obtained and no influence of skin type (Fitzpatrick I–VI [32]) on the measurements has been reported.

Numerous studies show the strong correlation between the carotenoid concentration in blood plasma and in the stratum corneum in human skin [8, 33–37] and in cow udder skin [38]. Thus, to determine the carotenoid concentration *in vivo*, non-invasive optical methods are widely used on the skin in dermatological and cosmetic research [6]. However, the carotenoid concentration in blood plasma increases and decreases much faster compared to skin due to the reservoir properties of the stratum corneum [39].

2.4 Measurement of Skin Carotenoids in Animals

The studies show that all types of stress factors have a negative effect on the antioxidant status of the human organism. The question therefore arose whether the non-invasive measurement of skin carotenoids can also be used to detect diseases in animals that cannot express themselves through language. In order to be able to measure the blood flow without interference from the field, the examination was carried out on the cow udder skin *in vivo* using non-invasive reflection spectroscopy of carotenoids.

First, it was investigated whether the antioxidant status of healthy animals, which are all fed the same diet, differs. It turned out that, regardless of the same approach conditions, the concentration of antioxidants in the animals differed depending on whether they were very calm or rather excited [10].

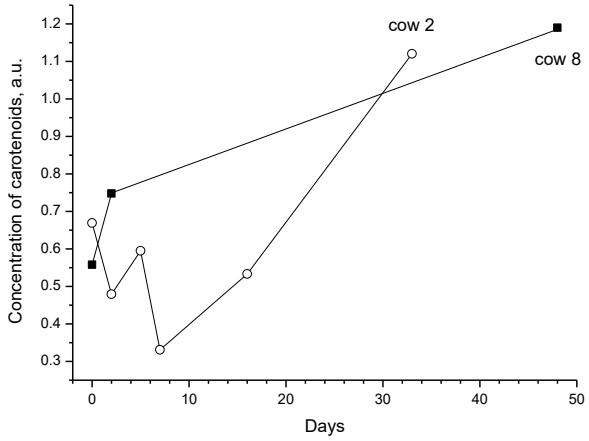

Fig. 2 Course of recovery of cutaneous carotenoid concentrations in two cows from the day of surgery (day 0) to the last measurement at the farm of origin. Cow 8 was discharged from the clinic two days post operation due to the favorable development of its condition, while cow 2 stayed in the clinic until day 16 post operation due to insufficient progress of its condition. The Fig. is adapted from Ref. [40].

Since it is not possible to scan large herds of cattle and wait for an animal to become ill, the reverse approach was used. In cooperation with the Veterinary Clinic of the Freie Universität Berlin, the antioxidant status of the skin in cattle was measured in cattle that suffered from a disease needing surgery. The measurements were taken directly after the operation and during its recovery process.

The course of recovery of cutaneous carotenoid concentrations in two cows from the day of surgery to the last measurement at the farm of origin is shown in Fig. 2.

This simple method is well suited to be integrated into the agricultural sector. This would make it possible to measure the animals every day and to detect a drop in antioxidants in the event of illness.

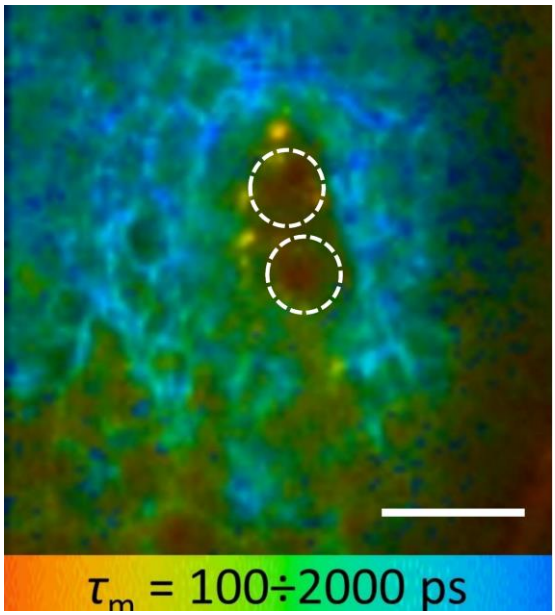

Fig. 3. TPT / FLIM image of the papillary human dermis *in vivo*. White dotted ovals show the areas with a short fluorescence lifetime, corresponding to blood capillaries (capillary loop). Pixel colors correspond to different values of the mean fluorescence lifetime τ_m in the 100–2000 ps range. Scale bar: 20 μm. The Fig. is adapted from Ref. [11].

2.5 Assessment of Blood Capillaries by Two-Photon Excited Fluorescence Lifetime Imaging in Human Skin

Different imaging methods are able to visualize blood circulation and the precise location of blood capillaries in the skin *in vivo* without the need of staining. Confocal laser scanning microscopy in reflection mode [11, 41–46] and optical coherence tomography [41, 45, 46] are the most commonly used methods being able to provide 3D images of the blood vessel network and even information on blood flow rates and velocity in the dermis. Two-photon tomography combined with fluorescence lifetime imaging (TPT/FLIM) could also be used for the visualization of blood capillaries in the dermis *in vivo*, where the low two-photon excited

autofluorescence intensity and short autofluorescence lifetime compared to other dermal components [47] are used as informative parameters [11]. The exemplary image of human skin papillary dermis is presented in Fig. 3. Blood capillaries (capillary loop) are clear visible as circle-like areas with a low autofluorescence intensity characterized with a short lifetime. It should be noted that one-photon excited FLIM measurement combined with flow cytometry of blood reveals the possibility to distinguish between exemplary cell types and blood plasma *in vitro* in a staining-free mode. Thus, white blood cell subtypes, such as eosinophilic and neutrophilic granulocytes, erythrocytes and blood plasma could be separated based on the FLIM data [48]. Erythrocytes exhibit low autofluorescence emission, that originates mainly from hemoglobin photo-products [49]. The cell separation in the blood is, however, currently not realized *in vivo* by measuring through the skin and these investigations are limited to *in vitro* studies using different optical methods [50–52].

3 Conclusions

Non-invasive methods are crucial in diagnostics and disease prevention. The use of a two-wavelength CO_2 laser is very well suited for the sensitive determination of the glucose concentration in blood samples. Here, a two-wavelength setting, where the first wavelength is in the absorption maximum of the glucose and the second wavelength is outside of this absorption range, is effective. Laser Doppler flowmetry is suited for the non-invasive determination of the influence of topically applied substances on blood flow. Using benzyl nicotinate, it was possible to differentiate between intercellular and follicular penetration pathways. It could be shown that the follicular penetration is an important route of penetration through the skin. Using Raman spectroscopy, it was possible to non-invasively detect carotenoid antioxidants in the blood plasma and in the skin. Similar results could also be obtained by reflection spectroscopy. Since the reflection spectroscopy-based device is small in size and stable to vibrations, it was used outside the laboratory on cattle. The aim was to be able to non-invasively assess the state of animal health via the skin's antioxidant status in order to be able to provide medical help quickly in the event of illness. This measuring method has proven itself for this task.

Autofluorescence lifetime measurements using two-photon tomography prove to be a sensitive method to visualize blood capillaries in the dermis *in vivo*, which is important for cosmetic and dermatological research. The experiments carried out provide clear evidence that non-invasive blood measurements are well suited to obtain information about physiological parameters of the organism and the skin.

Disclosures

The authors declare no conflict of interest.

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