FLUOROPHORES IN YOUNG HUMAN SKIN

MARCELA MORVOVÁ JR.[1], PETER JECZKO1, LIBUŠA ŠIKUROVÁ[1]

[1]*Department of Nuclear Physics and Biophysics, Faculty of Mathematics, Physics and Informatics of the Comenius University in Bratislava, Mlynska dolina F1, 842 48 Bratislava, Slovak Republic (Marcela.Morvova2@fmph.uniba.sk)*

Abstract: Nowadays characterization of specific fluorophores in biological samples has been well established and is routinely performed. Moreover *in vivo* fluorescence spectroscopy can also be applied when monitoring human skin. But it has not yet been published, if and to what extent at all, there are differences in genders and anatomical regions in skin fluorescence of healthy individuals. The primary goal of this study is to monitor differences in skin fluorescence which can relate to gender in young (aged 20 - 28) healthy individuals (n = 50). For this purpose we used *in vivo* fluorescence spectroscopy by Luminescence Spectrometer Perkin Elmer LS 45 and quartz optical fiber (Remote Fiber Optic accessory, Perkin Elmer). The detailed fluorescence data were scanned at an excitation wavelength range from 260 nm to 450 nm and the emission was taken in interval of 280 – 700 nm. In these broad wavelength intervals, three fluorescent bands were recognizable. The main fluorophores in human skin are: the aromatic amino acids tyrosine and tryptophan, pepsin digestible collagen cross-links (PDCCL), collagenase digestible collagen cross-links (CDCCL), elastin and porphyrins. The main significant gender differences were found in the amino acid region (excitation wavelength 280 nm, emission maximum at 340 nm).

Key words: fluorophores, human skin, autofluorescence.

Introduction

The use of fluorescence spectroscopy for characterization of specific fluorophores in biological samples has been well established and is routinely performed (Wagnieres et al. 1998). Moreover nowadays *in vivo* fluorescence spectroscopy find application even in monitoring of the human skin (Franco et al. 2016). It is known large number of fluorophores, which occur naturally in any biological tissue and are jointly responsible for the fluorescence properties of biological tissues. The fluorescence of endogenous fluorophores is often referred as autofluorescence.

Human skin perform weak autofluorescence based on native fluorophores with different concentration and spatial distribution within the skin (König a Riemann 2003; Bliznakova et al. 2007; Marcu et al. 2014). The main fluorophores in human skin are: the aromatic amino acids tyrosine and tryptophan, pepsin digestible collagen cross-links (PDCCL), collagenase digestible collagen cross-links (CDCCL), elastin, porphyrins (Franco et al. 2016; Stamatas et al. 2006; Kollias et al. 2002). All these fluorescence compounds are found in specific skin layers and often exhibit changes in fluorescence characteristics with modifications of skin functionality.

The existence of endogenous fluorophores presented in the human skin is currently well documented. In addition, many experimental studies revealed differences between skin autofluorescence of healthy individuals and individuals with skin disease. But it has not yet been published, if and to what extent at all, there are differences in genders and anatomical regions in skin fluorescence of healthy individuals. This study is therefore focus on gender differences in endogenous fluorophores in young human skin.

Materials and methods

In vivo fluorescence measurements were performed on a Luminescence Spectrometer LS 45 (Perkin Elmer) using quartz optical fiber (Remote Fiber Optic Accessory, PerkinElmer) with 9 mm opaque extension. Excitation-emission matrices (EEM) represent the relation among the fluorescence intensity, excitation wavelength and emission wavelength. They were created of the set of emission fluorescence spectra of samples with various excitation wavelengths.

The x-axis represents the emission wavelength and the y-axis the excitation wavelength. Fluorescence emission spectra were taken at excitation wavelengths from 260 nm to 450 nm in 10 nm step. Emission was taken in interval of 280 – 700 nm. Afterwards the single emission spectra were collected in accumulation mode (5 times), using the excitation wavelengths: 280 nm, 325 nm and 400 nm.
The young healthy adults (n = 50) both gender participate in this study. The volunteers were 20-28 years old with normal BMI in interval (18.5 – 24.5), didn't use any medication and had not any current medical problem. The study was performed during the winter season in Bratislava. The three anatomical regions were studied: *regio frontalis* (abbr. forehead), *regio dorsalis manus* (abbr. hand) and *regio brachii anterior – facies medialis* (abbr. arm).
Shapiro-Wilk W test was used for normal distribution testing. The comparison of variances was done using two-sided F test. The statistical significance of the differences was tested for an independent 2-population by an Unpaired t test or a Mann-Whitney U test. The level of significance was 0.05. The data are shown as a box plot, where the bottom and top of the box are the first and third quartiles, and the band inside the box is the second quartile (the median). The ends of the whiskers represent the minimum and maximum of all of the data.

Results and discussion

Typical fluorescence spectra of normal human skin in vivo are shown in Fig. 1 as a contour plot of excitation-emission matrix (EEM). In the EEMs of young healthy adults we can recognise three main fluorescent bands: (A) at the emission wavelengths of around 340 nm by excitation at 280 - 290 nm, (B) the emission wavelengths at about 390 nm by excitation at around 325 nm and (C) at approximately 600 nm emission by excitation at 400 nm. In the EEMs for normal healthy skin the band (A) at around 340/280 nm (peak emission/peak excitation wavelength) dominated with the highest intensity of skin autofluorescence.

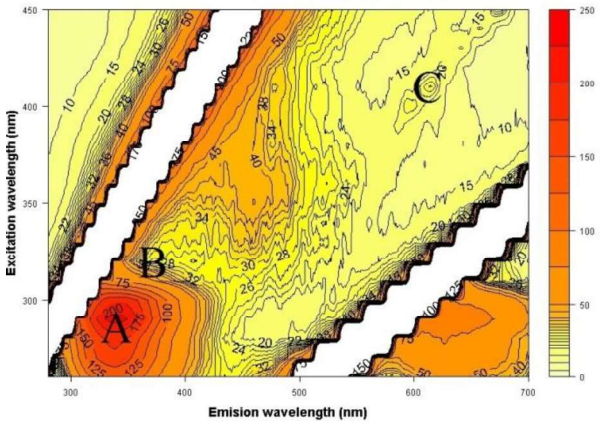


Fig. 1 Excitation-emission matrix of a representative normal human skin sample (men, 26, forehead) – *in vivo* measurement. The three main fluorescent bands can be recognised: (A) at the emission wavelengths of around 340 nm by excitation at 280 - 290 nm, (B) the emission wavelengths at about 390 nm by excitation at around 325 nm and (C) at approximately 600 nm by excitation at 400 nm.

Afterwards the single emission spectra of 50 healthy young adults both gender were analysed, using the excitation wavelengths: 280 nm, 325 nm and 400 nm. The three anatomical regions were studied: *regio frontalis* (abbr. forehead), *regio dorsalis manus* (abbr. hand) and *regio brachii anterior – facies medialis* (abbr. arm).
For the excitation 280 nm, the single emission spectra were collected from 310 nm to 400 nm (Fig. 2 left). The significant differences in the intensity of fluorescence signal were detected between genders (Fig. 2 right). The average fluorescence intensity in the emission maximum

at around 340 nm by excitation at 280 nm was 1.4 times higher in the group of men in comparison to the group of women in all three side of interest. Since radiation with a wavelength shorter than 300 nm does not propagate significantly into the dermis, the signal at 280 nm excitation mainly originates from the proteins located in the epidermis of human skin. In particular aromatic amino acids of proteins, mainly tyrosine and tryptophan contribute to fluorescence after 280 nm excitation (Franco et al. 2016; Kollias et al. 2002). We believe that for the higher skin autofluorescence detected in men is responsible differences in skin thickness and proliferation between the genders. The forehead has the highest fluorescence intensity from all three anatomical regions studied, probably due to frequent exposure to various environmental conditions including UV sun light.

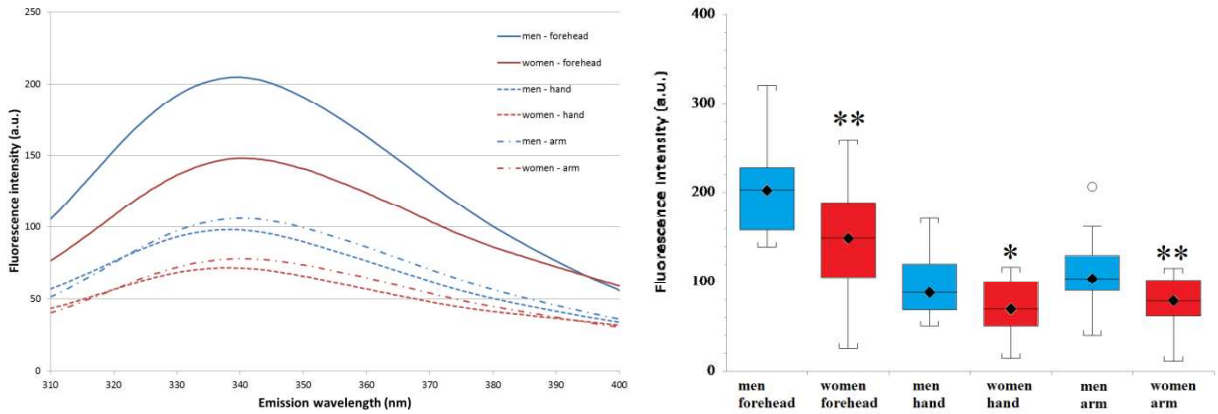


Fig. 2 On the left: The average from fluorescence intensities (280 nm excitation) of all data in the men group (blue line) and in the women group (red line) in all three anatomical regions studied (forehead, hand, arm). On the right: the box plot with median, first and third quartiles, minimum and maximum. * $P < 0.05$, ** $P < 0.005$

At the excitation 325 nm the single emission spectra were collected from 360 nm to 450 nm at which mainly the fluorescence maximum 390 nm is observed (Fig. 3 left). The fluorescence intensity at 390/325 nm in the men group was a little bit higher in all three anatomical regions than to the women group, although statistical analysis didn't verify significance of those differences (Fig. 3 right). Due to the fact that the 325 nm excitation can penetrate into the dermis (Franco et al. 2016), the primary endogenous fluorophores in dermis can be associated with this fluorescence, while the most common dermis fluorophore is collagen (Kollias et al. 2002; Gillies et al. 2000). It is interesting, that in 325 nm excitation the highest fluorescence intensity is in inner upper arm probably because thinness superficial skin layers in this region.

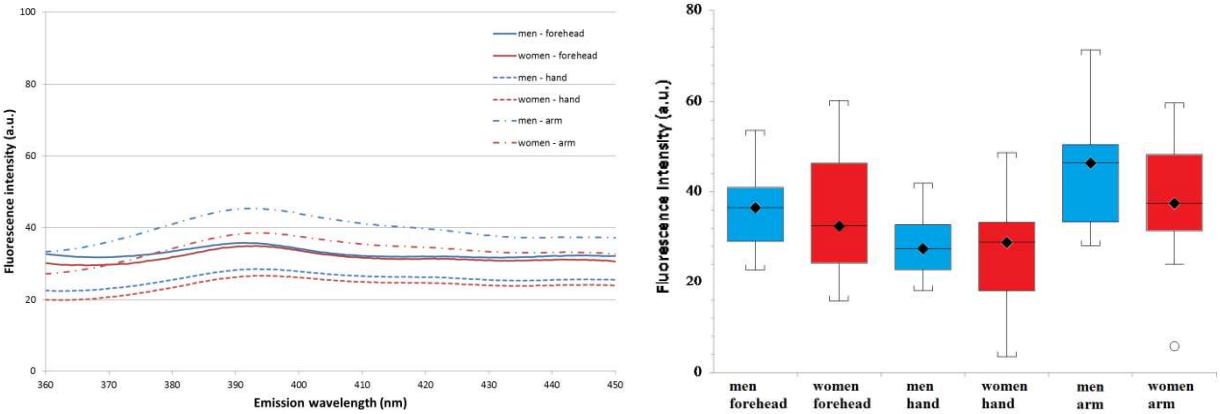


Fig. 3 On the left: The average from fluorescence intensities (325 nm excitation) of all data in the men group (blue line) and in the women group (red line) in all three anatomical regions studied (forehead, hand, arm). On the right: the box plot with median, first and third quartiles, minimum and maximum.

At the excitation 400 nm the single emission spectra were collected from 460 nm to 650 nm at which mainly the fluorescence maximum 600 nm was analysed (Fig. 4 left). In this excitation region the highest fluorescence intensity was observed in forehead (Fig. 4 right). No gender differences were noticed. This peak is generally assigned to endogenous porphyrins (König et al. 1993).

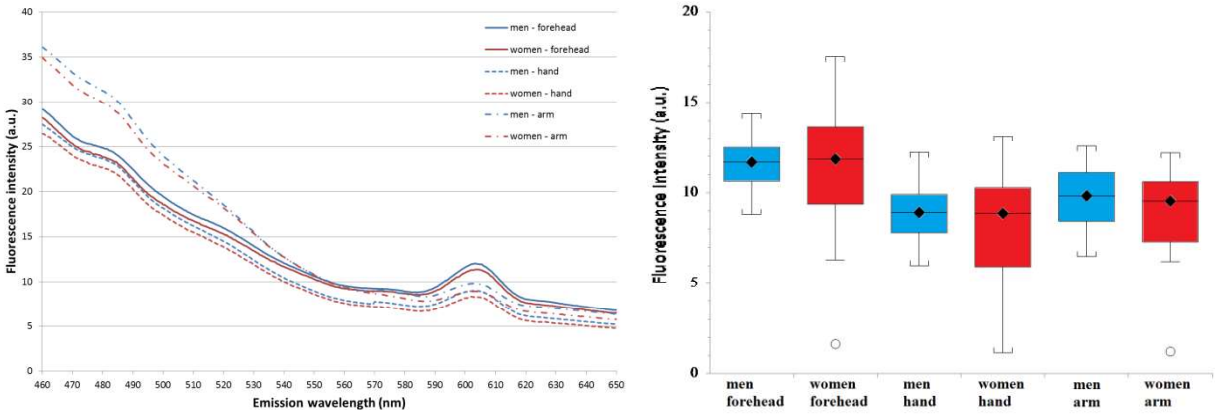


Fig. 4 On the left: The average from fluorescence intensities (400 nm - excitation) of all data in the men group (blue line) and in the women group (red line) in all three anatomical regions studied (forehead, hand, arm). On the right: the box plot with median, first and third quartiles, minimum and maximum.

Conclusions

In summary our investigation found that *in vivo* skin autofluorescence in young healthy adults show differences in gender and also anatomical regions studied (forehead, hand and arm). Mainly excitation 280 nm was characterized by an increase in the emission of amino acids in the men group compared to the women group. In 325 nm (collagen) and 400 nm (porphyrins) excitation the fluorescence intensity followed a similar pattern in men and women. The highest intensity of skin autofluorescence for the peaks 340/280 nm and 600/400 nm was reached on the forehead, whereas the 390/325 nm band showed the highest intensity on inner upper arm for both genders.

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References

BLIZNAKOVA, I, E BORISOVA a L AVRAMOV, 2007. Laser-and Light-Induced Autofluorescence Spectroscopy of Human Skin in Dependence on Excitation Wavelengths. V: *Proceedings of the International School and Conference on Optics and Optical Materials*. s. 1131–1136.

FRANCO, W, E GUTIERREZ-HERRERA, N KOLLIAS a A DOUKAS, 2016. Review of applications of fluorescence excitation spectroscopy to dermatology. *The British journal of dermatology* [online]. 2016, roč. 174, č. 3, s. 499–504. ISSN 1365-2133. Dostupné na: doi:10.1111/bjd.14221

GILLIES, Robert, George ZONIOS, R. ROX ANDERSON a Nikiforos KOLLIAS, 2000. Fluorescence Excitation Spectroscopy Provides Information About Human Skin In Vivo. *Journal of Investigative Dermatology* [online]. 2000, roč. 115, č. 4, s. 704–707. ISSN 0022202X. Dostupné na: doi:10.1046/j.1523-1747.2000.00091.x

KOLLIAS, Nikiforos, George ZONIOS a Georgios N. STAMATAS, 2002. Fluorescence spectroscopy of skin. *Vibrational Spectroscopy* [online]. 2002, roč. 28, č. 1, s. 17–23. ISSN 09242031. Dostupné na: doi:10.1016/S0924-2031(01)00142-4

KÖNIG, Karsten, Herbert MEYER, Herbert SCHNECKENBURGER a Angelika RÜCK, 1993. The study of endogenous porphyrins in human skin and their potential for photodynamic therapy by laser induced fluorescence spectroscopy. *Lasers in Medical Science* [online]. 1993, roč. 8, č. 2, s. 127–132. ISSN 02688921. Dostupné na: doi:10.1007/BF02547809

KÖNIG, Karsten a Iris RIEMANN, 2003. High-resolution multiphoton tomography of human skin with subcellular spatial resolution and picosecond time resolution. *Journal of biomedical optics* [online]. 2003, roč. 8, č. 3, s. 432–9. ISSN 1083-3668. Dostupné na: doi:10.1117/1.1577349

MARCU, Laura, Paul M. W. FRENCH a Daniel S. ELSON, 2014. *Fluorescence lifetime spectroscopy and imaging : principles and applications in biomedical diagnostics* [online]. B.m.: CRC Press. ISBN 9781439861677. Dostupné na: doi:10.1201/b17018

STAMATAS, G N, R B ESTANISLAO, M SUERO, Z S RIVERA, J LI, A KHAIAT a N KOLLIAS, 2006. Facial skin fluorescence as a marker of the skin's response to chronic environmental insults and its dependence on age. *The British journal of dermatology* [online]. 2006, roč. 154, č. 1, s. 125–32. ISSN 0007-0963. Dostupné na: doi:10.1111/j.1365-2133.2005.06949.x

WAGNIERES, Georges A., Willem M. STAR a Brian C. WILSON, 1998. In Vivo Fluorescence Spectroscopy and Imaging for Oncological Applications. *Photochemistry and Photobiology* [online]. 1998, roč. 68, č. 5, s. 603–632. ISSN 00318655. Dostupné na: doi:10.1111/j.1751-1097.1998.tb02521.x