IN VIVO STUDY OF THE HUMAN SKIN BY THE METHOD OF LASER-INDUCED FLUORESCENCE SPECTROSCOPY

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INTRODUCTION

The goals of this study are to perform a preliminary evaluation of the diagnostic potential of noninvasive laser-induced autofluorescence spectroscopy (LIAFS) for human skin *in vivo* and optimize of detection and diagnosis of hollow organs and skin.

Optical methods have a important role in investigation of physiological processes in human body. Spectroscopy has the potential to provide important diagnostic information about tissue. Light signals can be delivered and collected via optical fibers, so all parts of the body can be accessed through endoscopes. The diagnosis is noninvasive, i.e. with low-intensity laser, that it does not change characteristics of tissue. For diagnostic goals we use laser-induced fluorescence (LIF) of the tissues.

In recent years, there has been growing interest in the use of laser-induced fluorescence to discriminate disease from normal surrounding tissue. The most fluorescence studies have used exogenous fluorophores of this discrimination. However, laser-induced autofluorescence (LIAF) which is used for diagnosis of tissues in the human body avoids administration of any drugs.

In this study is presented a technique for optical biopsy of *in vivo* human skin. The autofluorescence characterization of tissue relies on different spectral properties of tissues. It was demonstrated a differentiation between normal skin and skin with vitiligo.

Two main endogenous fluorophores in the human skin account for most of the cellular autofluorescence for excitation wavelength 337 nm: reduced form of nicotinamide adenine dinucleotide (NADH) and collagen. The autofluorescence spectrum of human skin depend on main internal absorbers which are blood and melanin. In this study was described the effect caused by blood and melanin content on the shape of the autofluorescence spectrum of human skin. Human skin fluorescence spectrum might provide dermatologists with important information and such investigations are successfully used now in skin disease diagnostics, in investigation of the environmental factor impact or for evaluation of treatment efficiency.

Description of the LIF spectrofluorometer

A real-time, noninvasive spectrofluorometer have been developed and tested in the Institute of Electronics of BAS. The system can be used *in vivo* for the detection and diagnosis of cancer and other tissue pathologies. It is based on the interaction of light with tissue and is therefore called the optical biopsy system. Spectra of different parts of the human body can be collected allowing to be examined rapidly and without any damage to the tissue.

The LIAFS experimental setup consist of excitation source -nitrogen laser (ILGI-503) emitting at 337 nm (10^{-9} s pulse duration, 10 Hz repetition rate, 14 μJ), optical fibers, one of them delivered exciting light and other collected autofluorescence signal, sample, and detector (S2000, "Ocean Optics"). The fluorescence light dispersed by the spectrometer was detected by CCD-array detector composed of 2048 pixels, interfaced to an optical multichannel analyzer (OMA). The spectral resolution is approximately 8.5 nm per pixel. This system is control by a computer that allow data storage and spectral display.

The system utilizes a optical fiber probe which can be passed down an endoscope and into contact with tissue to be examined, so all parts of the body should be accessible by this method. Exciting light is delivered to the tissue via one or more optical fibers in this probe. After traveling through the tissue, light is collected by a another optical fiber and analyzed.

The system can be used in real-time for the diagnosis *in vivo* of researching tissues. The development of such devices is of major importance as they allow the optimization of photodetection of the tissues.

Experimental results

In this study is demonstrated a spectral differentiation between normal skin and skin with vitiligo.
Skin tissue consists of many kinds of fluorophores with different excitation and fluorescence spectra, different quantum efficiencies, which have not been completely studied. Among the many endogenous skin fluorophores being investigated, the wildly distributed are the different forms of NAD located in the epidermis and dermal collagen. Approximately 75% of the dry weight of the dermal tissue is composed of collagen fibers. Collagen is the main structural component of skin tissues and accounts for about 90% of protein in human dermis. Collagen fibers show a constant density throughout all dermal layers. Exciting spectrum of the collagen is in the region 340-360 nm and fluoresces in the region 400-500 nm. Reduced (NADH) and oxidized (NAD+) forms of nicotinamide adenine dinucleotide take part in the cellular energy metabolism, and the intensity of the specific fluorescence may be used for diagnostics of the metabolism disfunction. NADH is excited in the region of 360 nm and fluoresces in the region 400-500 nm.
Autofluorescent spectra were recorded from several volunteers from normal skin and skin with vitiligo. Autofluorescent spectrum of normal skin is shown in figure 2.
There has a decrease of the autofluorescent signal in case of increase of the melanin content in normal skin (fig.2(a)).
Vitiligo is a common disease with loss of normal melanin pigments and functioning melanocytes from otherwise healthy looking skin. Decrease in the melanin content within the skin tissue leads to increase of autofluorescence intensity of collagen and NADH. In the case of the missing melanin the intensity of the autofluorescence should be maximum. Autofluorescent spectrum of skin with vitiligo is shown in figure 3.
In the spectrum on the fig.3 the autofluorescent signal of the skin increased, but the band of NADH fluorescence (max 440 nm) decreased in the spectrum of skin with vitiligo.

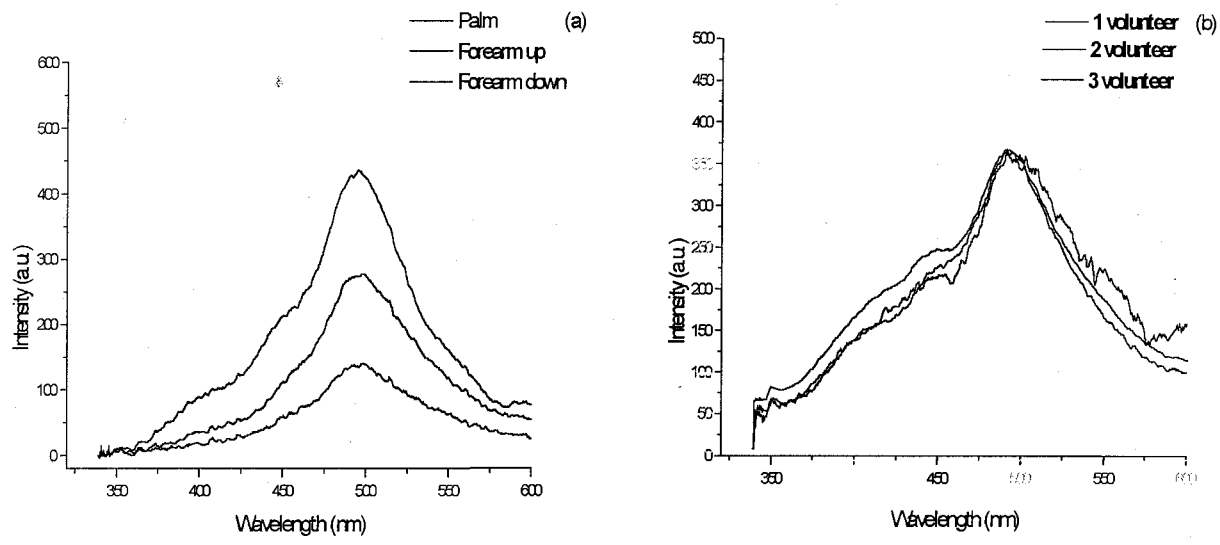

Fig.2 (a) Autofluorescence spectra of normal skin from different areas of one hand;
(b) Autofluorescence spectra of normal skin from different people

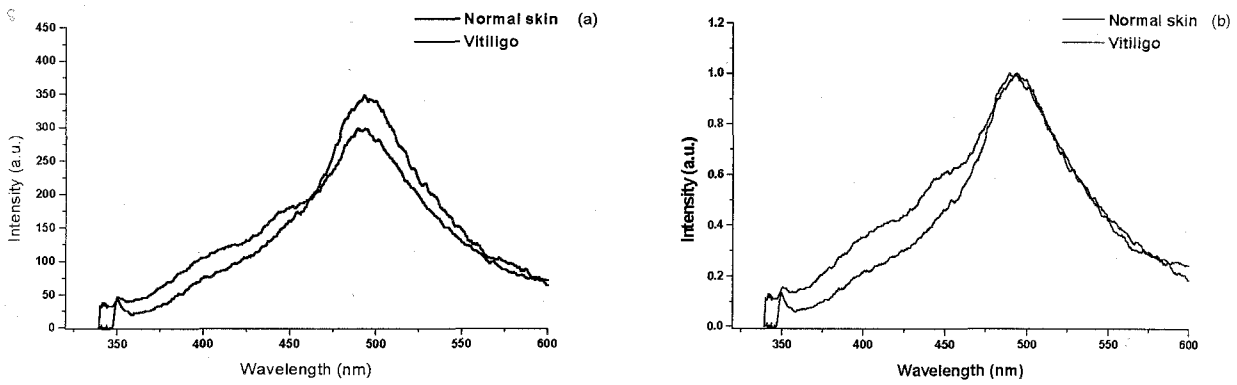

Fig.3 (a) autofluorescent spectrum of skin with vitiligo;
(b) normalized autofluorescent spectrum of skin with vitiligo

Discussion

Vitiligo is a common idiopathic acquired or inherited disease with loss of normal melanin pigments and functioning melanocytes from otherwise healthy looking skin.

The melanin is product of the methabolism of the fenilalanine and tyrosin. The fenylalanine turns into tyrosine under influence of enzim fenyl alanine hidrolaza, NAD(P)H and three molecules oxygen. In the melanocytes the tyrosine turns into the main pigment of the body - melanin. If one of the catalisators is missing the conversion can not complete. There is possible that NADH content decrease in the damaged areas in the skin with vitiligo. We note that in the autofluorescent spectra.

In the human body NAD is a result of the metabolism of tryptofane. Only 2 % of the tryptofane turn into NAD which is not sufficient to satisfy the need from nicotinamide like a vitamine (between 10 and 20 mg daily). The main way for receiving of nikotinamide is through the food. The decrease of the NADH content disturbs many of the general functions of the human body.

The collagen content, which is a main structural unit of the skin doesn't change because there hasn't changes in the structure of the skin with vitiligo.

Decreasing of the autofluorescent signal in the region of 420 - 460 nm of the skin with vitiligo means that there is a decrease of the content of NADH in the damaged area. It is possible that the low content of NADH can be the cause of appearance of this condition of disorder. In this case it should search for additional sources of nicotinamide from different medicine or foods.

On the basis of received autofluorescent spectra from different patients with vitiligo a suggestion was made that a depigmentation of the human skin is associated with the losses of NADH.

CONCLUSIONS

A real-time, noninvasive spectrofluorometer for optical biopsy have been developed and tested in the Institute of electronics of the Bulgarian Academy of Sciences.

On the basis of received autofluorescent spectra from different patients with vitiligo a suggestion was made that a depigmentation of the human skin is associated with the losses of NADH.

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