Application of fluorescence technique in studying facial skin

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ABSTRACT

This study introduces using simple optical system for fluorescence stimulation by UVA light to obtain in vivo facial skin fluorescence images. The red fluorescence was observed on normal skin and skin with comedones, while inflamed facial skin emitted the blue light. The fluorescence spectra were measured for determining the origination of facial red fluorescence. With an emission peak at the wavelength about 630 nm, the measured spectra showed that protoporphyrin IX responds to red color in facial skin. Analysis of the fluorescence imaging and spectra can give information about the disease state of skin and can be used for designing non-invasive optical devices for skin diagnosis.

Key words: *facial skin, fluorescence, porphyrin, protoporphyrin IX.*

1. INTRODUCTION

In recent years the number of people suffering from skin diseases generally and acne particularly has extremely increased due to the environmental pollution, the widespread utilization of chemicals in agriculture and food industry, etc., especially in Vietnam with the tropical climate and the major population working in the agricultural sector. Some traditional devices have usually been used in the diagnosis of skin diseases, such as facial lamp or microscope for skin surface examinations but they are not always effective, especially in early-stage diagnosis of skin disease. Recently, some new tools have been developed such as fiber-based fluorimeters – SkinScan system (Jobin Yvon, France), where fluorescence of endogenous aminoacids is used for cutaneous lesions investigations, or DYADERM system (Biocam GmbH, Germany), which is applied for photodynamic diagnosis with exogenous photosensitizers [1]. However, up to our days there is no simple clinical device, based on autofluorescence detection of skin surface, which could be used as an universal tool for early stage detection of skin diseases.

This investigation is a part of clinical trial for developing mentioned skin diagnostic device based on fluorescence imaging. In this paper, using light-emitting diodes operating in the near ultraviolet spectral regions (UVA), fluorescence imaging and spectroscopy techniques were incorporated into studying fluorescence

properties of normal skin, skin with comedones and inflamed facial skin. The normal skin and comedones emitted the red fluorescence, while inflamed skin emitted the blue one. The fluorescence spectra were measured for determining the origination of facial red fluorescence.

2. MATERIALS AND METHODS

2.1. Subjects

Thirty volunteers of both sexes and various ages (age range 15-30 years, 15 females and 15 males) were included in this study. Among them, there are 5 volunteers with normal skin and 25 others with mild to moderate acne. The skin surface was wiped with alcohol before photography.

2.2. The optical systems

For studying the facial fluorescence properties, the majority of researchers have used lasers or xenon lamp for exciting samples due to its advantage of high efficiency [2-5]. However, the purpose of this research is designing a portable, compact and inexpensive skin diagnostic tool, for that the lasers or xenon lamp are not suitable. In the previous study [6] we had tested the power LEDs emitting 380-nm peak in exciting facial fluorescence. The results showed that the high fluorescence intensity of all obtained images is available for normal observation in daylight condition. Therefore, the 380-nm LEDs were chosen in this study.

The fluorescence imaging was obtained by using the optical system shown schematically in Figure 1(a): sample (1), 10x-magnification system (2), DSLR camera (3), UV filter (4), LED (5), power source (6). The light source was a 380-nm LED driven by a stabilized power source (Agilent/HP 6632B 20V-5A Power Supply). An UV bandpass filter (UG-1, Edmund Optics) allows the transmission only UV light and eliminates other visible light from the LED. A 10x-magnification multiple lens system was used for magnifying fluorescence images taken by a camera (Canon DSLR camera 550D). The measured areas were classified in two zones: T-zone (the forehead, nose and chin) and the U-zone (both cheeks).

Model of fluorescence spectroscopy instrumentation is presented in Figure 1(b): sample (1), optical fiber (2), monochromator (3), computer (4), UV filter (5), LED (6), power source (7). The 0.25m monochromator (Newport MS257) is connected to the computer to register the spectrum using specialized software.

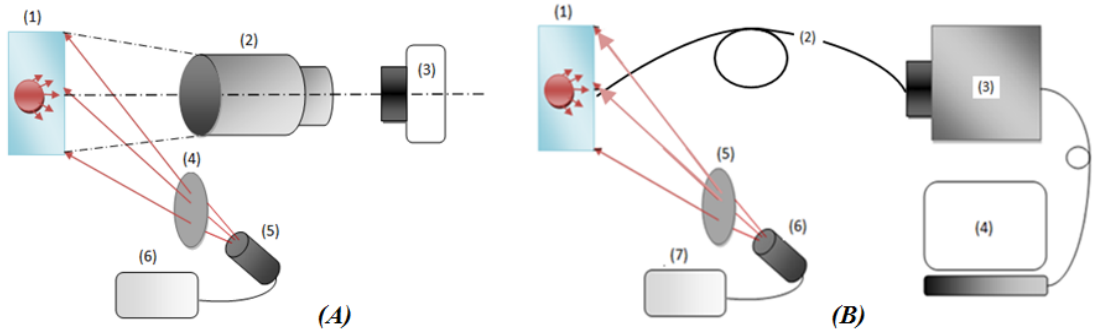


Fig. 1. Experimental setup used for fluorescence photography (A) and fluorescence spectroscopy (B).

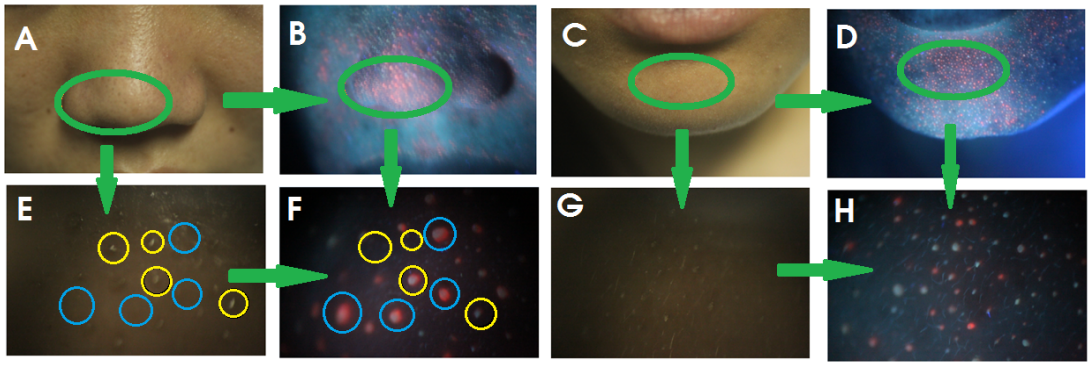


Fig. 2. Fluorescence photographs at nose and cheek areas under white light and UVA (A, B, C, D), and 10x magnification respectively (E, F, G, H)

3. RESULTS

3.1. Fluorescence imaging

3.1.1. The red fluorescence

Figure 2 shows the facial fluorescence images of a volunteer with normal skin. Under UVA excitation, the red color appeared in the areas of nose, chin, forehead and cheek. For detailed observation, the strong emission areas (green ovals) were magnified 10 times (Fig. 2E-2F-2G-2H). At the magnified nose area (Fig. 2E-2F), we can see comedones (yellow circles) not always emitted the weak red fluorescence signals, but some areas with no mark of acne (blue circles) even emitted the stronger red color than the comedones. The same thing was observed at the cheek area (Fig. 2G-2H).

Many researches have showed the direct correlation of red fluorescence with P. acnes [2, 7, 8]. Acne is a chromic inflammatory disorder of the pilosebaceous follicles with a multifactorial etiology and pathogenesis. The follicular impactions develop into initially invisible lesions (microcomedones) and then into clinically evident comedones. Microcomedones and comedones (black- and whitehead) are suitable microenvironment for colonization by cutaneous bacteria, especially P. acnes. These bacteria produce proinflammatory mediators and free fatty acids, which are responsible for the appearance of inflamed acne lesions [9]. Porphyrins (coproporphyrin) are further endogenous metabolic products of Propionbacteria, which might additionally contribute to the perifollicular inflammatory reaction. Porphyrins are native fluorophores and strongly fluorescent. Their presence can be demonstrated by orange-red fluorescence with peak at 618 nm in the follicle openings by examining facial skin under UVA light.

However, as can be seen in Figure 2F, the normal skin without comedones also emitted red fluorescence. The other studies have showed that another porphyrin encountered in skin is protoporphyrin IX (Pp IX) which is produced by the body in a pathway for biosynthesis of heme. Protoporphyrin IX also produces fluorescence in the red spectral region with peak at 635 nm [10].

From above mentioned research results, the red fluorescence in Figure 2 is possible to have the correlation with the presence of coproporphyrin or protoporphyrin IX. This supposition will be tested by measuring the fluorescence spectra of this sample in the next subchapter.

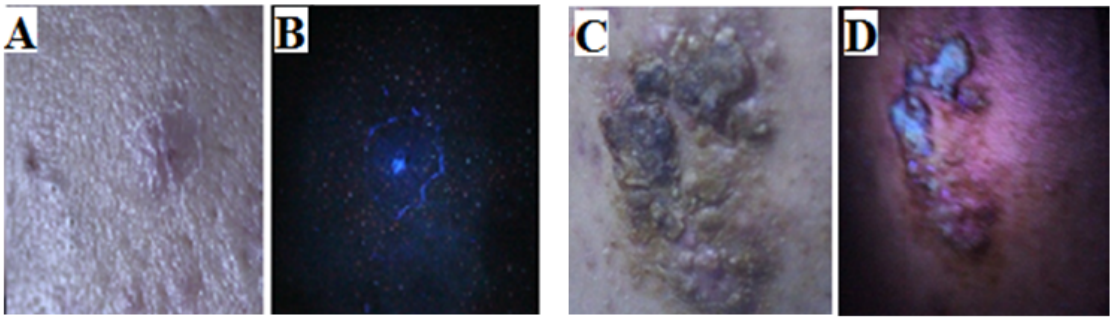


Fig. 3. Examples of fluorescence images in the inflamed skin under white light (A, C) and 380 nm (B, D)

3.1.2. The blue fluorescence

In this research, the red fluorescence was found in normal skin and comedones, while the blue fluorescence was observed only in the inflamed skin (Fig. 3).

Dermatologists classify the types of acne into four grades. Determining acne grade is done by visual inspection of the skin. Specific criteria are used to classify acne symptoms, including: grade 1 - mild and non- inflammatory acne; grade 2 - moderate and slight inflammatory acne; grade 3 - considered severe acne with more amount of inflammation; grade 4 - pronounced amount of inflammation and breakouts are severe.

Inflammation is a process by which the white blood cells and chemicals protect the body against the infection caused by bacteria and viruses. White blood cells, or leukocytes, are the cells of the immune system that are involved in defending the body against both infectious disease and foreign materials. There are five types of leukocytes, among them several types (including monocytes and neutrophils) are phagocytic. When inflammation occurs, chemicals from the body's neutrophils are released into the blood or affected tissues to protect your body from foreign substances. Monici M. et al. [11] studied the fluorescence property of neutrophil granulocytes and reported that neutrophil excited by 366 nm and 436 nm wavelength emits wide fluorescence spectrum in visible range, with high intensity at 450 nm (blue light) and 520 nm (green light), respectively.

In this research, inflammatory acnes were excited by 380 nm and emitted the blue fluorescence. We suppose the nature of the blue fluorescence is neutrophil. Neutrophils appear only in the area of inflammatory acnes and that is why the blue fluorescence was obtained only in inflamed skin.

3.2. Fluorescence spectra

Most studies of the facial fluorescence have used fluorescence imaging and fluorescence spectroscopy separately. The application of both qualitative (imaging) and quantitative (spectroscopy) methods can give a full overview about the origin of the red facial fluorescence.

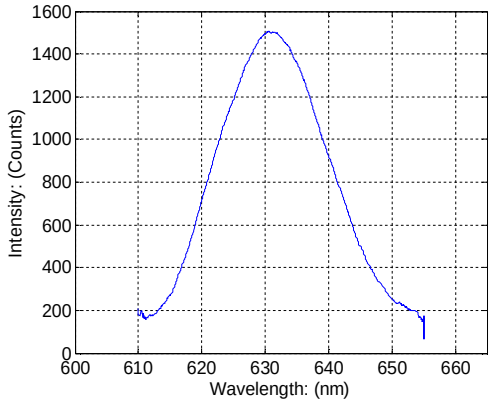
From above mentioned studies, we know that coproporphyrin and protoporphyrin IX can emit the red fluorescence at 618 nm and 635 nm respectively. However, due to its proximate maxima in the spectral region of 620–635 nm, the fluorescence imaging lacks specificity in distinguishing coporoporphyrin from PpIX. In addition, when fluorescence spectroscopy is applied to human skin in vivo, the detected fluorescence spectrum is a resultant of

modulation by the characteristic absorption of skin chromophores, such as blood and melanin. Thus additional procedure is required for the quantification of both porphyrins in the fluorescence spectroscopy measurements [10].

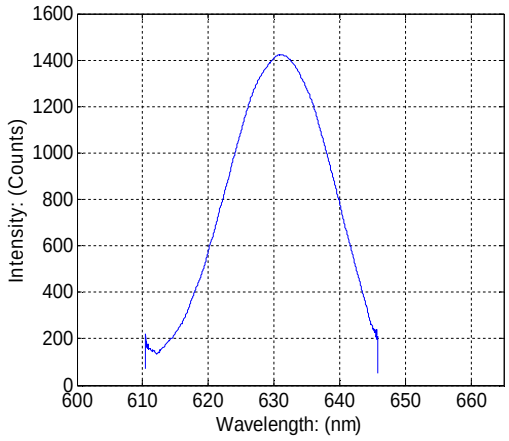
In the present work, the nose and cheek areas have showed the emission spectra with the same shape and the peaks at about 630 nm, which is due to the presence of PpIX (Fig. 4). As known, the intensity of peaks depends on the density of the fluorophores. In this case, the different fluorescence intensities of the nose cheek areas can give information about the density of PpIX.

From mentioned result we can see that the fluorescence spectroscopy has ability to determine the origin of the red facial fluorescence and this will be necessary to detect the presence of P.acne and to completely diagnose the disease state of skin. Further investigation is required for additional cases with and without comedones to study the correlation between coproporphyrin and protoporphyrin IX in red facial fluorescence.

The fluorescence spectra of blue facial fluorescence of inflammatory acne has not been measured due to its low intensity. The optical system used for measuring the fluorescence spectra (Fig. 1B) requires further improvement for ability of weak fluorescence signal measurements.



(A)



(B)

Fig. 4. Fluorescence spectra of nose (A) and cheek (B).

4. CONCLUSION

We have determined the origination of red facial fluorescence by fluorescence spectra measurement. With one emission peak at about 630 nm, the result showed that protoporphyrin IX is responsible for the red fluorescence in normal skin. The blue fluorescence was observed only in the inflamed skin. We suggest that the blue fluorescence is caused by neutrophils appearing in the area of inflammation. The nature of facial acne fluorescence and its specification requires further investigation to improve the design of non-invasive optical device for skin disease diagnosis.

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Ứng dụng kỹ thuật huỳnh quang trong nghiên cứu da mặt

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TÓM TẮT

Nghiên cứu này giới thiệu việc sử dụng một hệ thống quang học đơn giản kích thích huỳnh quang bằng ánh sáng cực tím để thu được hình ảnh huỳnh quang da mặt. Sự phát huỳnh quang màu đỏ đã được quan sát đối với da bình thường và da mụn, trong khi da mặt bị viêm phát ra huỳnh quang màu xanh. Quang phổ huỳnh quang trên đã được đo để xác định nguyên do của huỳnh quang màu đỏ trên da mặt. Với một đỉnh phát xạ ở bước sóng khoảng 630 nm, quang phổ đo được cho thấy protoporphyrin IX là nguyên nhân của huỳnh quang đỏ trên da mặt. Phân tích các hình ảnh huỳnh quang và quang phổ của chúng có thể cung cấp thông tin về tình trạng bệnh của da và có thể được dùng để thiết kế các thiết bị quang học không xâm lấn để chẩn đoán da.

Từ khóa: *da mặt, huỳnh quang, porphyrin, protoporphyrin IX*

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