Endogenous Skin Fluorescence *In Vivo* on Human Skin

Introduction

The fluorescence spectra of intrinsic protein fluorophores have been studied extensively and used in investigating biological events. Native fluorophores in human tissue include NADH, collagen, elastin, tryptophan, tyrosine, porphyrins and FAD. Recent studies have proven that skin fluorescence originating from tryptophan and collagen can serve as a quantitative marker for normal, diseased, chronically-aged, and sun-damaged skin. Characterizing fluorescence spectra of these native fluorophores also allows the study of changes in these markers in both healthy and diseased skin.

The HORIBA Jobin Yvon Fluorolog® spectrofluorometer can measure endogenous fluorescence *in vivo* on human skin. When configured with double-grating monochromators at both excitation and emission, the instrument provides extremely high rejection of stray light. A fiber-optic bundle accessory transmits light to the skin and returns the fluorescence emission to the detector. The Fluorolog® is capable of *in-vivo* monitoring of endogenous skin fluorescence from a highly scattering background such as human skin, with high sensitivity and precision.

Experimental method

Fluorescence spectra were measured *in vivo* on human skin. As shown in Fig. 1, light from a Xe lamp was passed through a double-grating monochromator, where the selected wavelengths were focused into one arm of a randomized, bifurcated fiber-optic bundle. This light traveled to the common end of the bundle irradiating skin in contact with the bundle. The emitted light was then collected by the emission bundle, passed through another double-grating monochromator, and finally directed to the emission photomultiplier-tube detector. The probe was held by an adapter to ensure good contact and even pressure between the bundle and the skin.

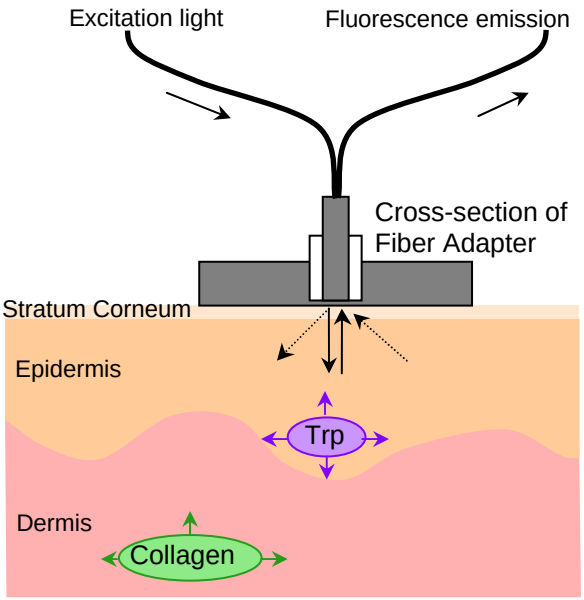

Fig. 1. *Diagram of skin layers and light path during a fluorescence excitation or emission scan.*

Three excitation spectra were collected from human skin. The first excitation spectrum was scanned from 260–320 nm with the emission monochromator parked at 340 nm, the second spectrum from 260–380 nm with the emission parked at 400 nm, and the third spectrum from 260–420 nm with the emission set to 440 nm. The band-

pass of the instrument was set to 5 nm for both excitation and emission, and the integration time was 0.2 s.

Results and discussion

Fig. 2 shows three excitation fluorescent spectra from normal healthy skin. These spectra are differentiated by three peak maxima: 295 nm, 340 nm, and 360 nm. The excitation spectrum with a peak maximum at 295 nm originated from tryptophan fluorescence in the epidermis. The excitation spectrum at 340 nm corresponds to the pepsin-digestible collagen cross-links' fluorescence, and the 360-nm band is the collagenase-digestible collagen cross-links' fluorescence.[1]

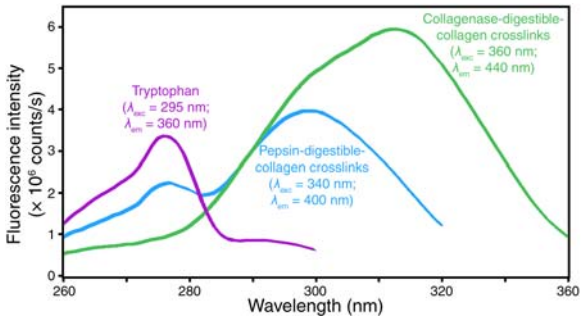

Fig. 2. *Fluorescence-excitation spectra of endogenous human skin* in vivo*.*

Fluorescence spectra of tryptophan and collagen can serve as quantitative markers for photoaging and natural aging. Exposure to UV light causes significant qualitative and quantitative changes in cutaneous fluorescence spectra. In photoaged skin, the tryptophan signal (λ_{exc} = 295 nm) increases with photoaging and the two distinct collagen bands became one broad band, centered at 355 nm. In addition, two new bands, i.e., excitation at 270 and 350 nm, were observed in the photoaged skin. In contrast, the tryptophan band decreases with natural aging while the 335–340-nm band corresponding to the pepsin-digestible collagen cross-links increases. The 360-nm band of the collagenase-digestible collagen cross-links fluorescence remains the same with natural aging.

Conclusions

Fluorescence spectroscopy has demonstrated a way to characterize and quantify skin aging and photoaging. The Fluorolog® allows direct *in-vivo* monitoring of endogenous skin fluorescence, which makes it possible to follow markers (tryptophan and collagen) in skin, and their changes in both healthy and diseased tissue. It also provides the possibility of evaluating the effect on skin from the modifications of these native proteins or the induction of exogenous chromophores. The Fluorolog® will find a wide range of applications in skin research and photodynamic therapy.

[1] Kollias, N.; Gillies, R.; Moran, M.; Kochevar, I.E.; Anderson, R.R. *J. Investig. Dermatol.*, **1998**, *111*(5), 776–780.

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