Skin autofluorescence photo-bleaching and photo-memory

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ABSTRACT

Photo-bleaching of in-vivo skin autofluorescence intensity under continuous low power laser irradiation has been studied. Temporal behavior of single-spot fluorescence and spectral fluorescent images have been studied at continuous 405 nm, 473 nm and 532 nm laser excitation and/or pre-irradiation, with power densities well below the laser-skin safety limits. Skin autofluorescence photo-memory effects (laser signatures) have been observed and analyzed, as well.

Keywords: in vivo skin, photo-bleaching, autofluorescence.

1. INTRODUCTION

Photo-bleaching is a process of fluorescence intensity decrease during a lasting optical irradiation. The skin autofluorescence photo-bleaching (AFPB) was examined several times in the recent years, and double-exponential intensity decrease has been observed in most cases. AFPB has been recorded in a wide range of power densities (1-500 mW/cm²) at various laser excitation wavelengths - ultra-violet (337 nm), violet (405 nm), blue (442 nm), green (532 nm) and red (632 nm), both under continuous and pulsed excitation [1-3].

Skin fluorescence photo-bleaching has a potential for clinical applications in photodynamic therapy, skin keratinoid concentration determination, sensitizing agent controlling, etc. [4-8]. Mechanism of the skin AFPB effect has not been explained in details so far, but several hypotheses are examined experimentally. Low power laser irradiation may cause direct skin fluoroflore (for instance, porphyrin) degradation, as well as modification of absorption and quenching properties; photo-initiated cellular processes cannot be excluded, too.

One should note that AFPB of healthy skin takes place at very low laser power densities, even lower than the safety limits fixed for skin at the European laser safety standard (200 mW/cm², exposition time up to 10³ s) [8].

This study continues our previous research [9-11] with the aim to understand more deeply the in-vivo skin AFPB effects under low-power cw-laser irradiation at four visible wavelengths – 405 nm, 473 nm, 532 nm and 634 nm.

2. METHODS AND EQUIPMENT

Two methods of skin autofluorescence recording were used in the study: the single-spot irradiation/detection by means of a fiberoptic probe, and the spectral imaging method.

2.1 Single-spot AF excitation and detection

The single-spot measurement setup scheme is shown in Fig. 1. The setup comprised four cw lasers emitting at 405 nm (BWB-405-40-PIG-200-0.22-SMA, B&W Tek, USA), 473 nm (MBL-III-473nm-30mW, CNI, China) and 532 nm (BWT-532-15-SMA, B&W Tek, USA) with silica core optical fiber output via the SMA-connector. The excitation fiber represented one leg of a Y-shaped optical fiber bundle (FRC-7UV400-2-ME, Avantes, The Netherlands) comprising 6 more fibers for delivery of the fluorescent light to spectrometer (AvaSpec-2048, Avantes, The Netherlands) which was connected to PC.

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The fibreoptic probe was sloped at 45 degree angle relatively to the skin surface and placed ~3 mm from it; long pass filters were used for laser-blocking prior to spectrometer. The typical duration of excitation was 3-5 minutes, the spectrometer integration time 0.5 sec, laser power density on the skin 30...85mW/cm², area of the excited skin surface ~10 mm² [9]. During the skin AF recovery measurements, repeated 532 nm laser excitations for periods of 1 minute were used to bleach the autofluorescence on forearm, with 1 minute intervals between the excitations. Overall 3 volunteers of skin photo type III and IV participated in the trial. In the studies of skin photo-memory, additionally 405 nm, 473 nm and 634 nm laser pre-irradiation and/or AF excitation took place at power densities of ~35 mW/cm².

Fig. 1. Setup scheme for single-spot detection of the skin AFPB.

2.2 Spectral imaging of the skin autofluorescence

To follow the changes in autofluorescence intensity after the laser induced photo-bleaching, a multispectral imaging system shown in Fig. 2 was used. The setup comprised a cw laser for irradiation of skin area of ~30-100 mm² via a single-fiber cable, and the multispectral camera (Nuance 2.4 N-MS-EX, Cambridge Research and Instrumentation, USA) with tunable narrow-band liquid crystal filter. A mask of non-transmitting material was imposed to cover a half of the selected skin area, and the uncovered region was pre-irradiated by a laser with the power density of 50-85 mW/cm² for ten minutes. After one minute the mask was removed and skin autofluorescence from the whole target area was recorded. To acquire fluorescence images, narrow-band 10 nm filtering at the spectral range corresponding to the respective skin autofluorescence maxima at each excitation wavelengths were used.

Fig. 2. Setup scheme for spectral imaging of skin autofluorescence.
3. RESULTS AND DISCUSSION

Fig. 3 presents the healthy skin AF intensity decay detected simultaneously at two wavelengths - AF band maximum 600 nm and 680 nm - under continuous 532 nm laser excitation. More rapid bleaching at 600 nm was observed. The same tendency was observed if other AF wavelength pairs were compared – the shorter wavelength of AF spectra exhibited faster photo-bleaching.

The AFPB effect of healthy skin in conditions when the same place was repeatedly excited by stabilized 532 nm cw-laser is illustrated at Fig. 4. The fluorescence intensity exponentially decreased during irradiation periods; however, after the „rest” between two excitations the AF intensity did not return back to the initial level, but was just slightly higher than that at the moment of switching off the previous irradiation. This indicates to photo-induced irreversible processes in skin which can be regarded as “photo-memory” of skin. Our previous study [10] showed that it is a long-term effect – the AF intensity recovered to ~80% of its initial level only after three to five days. If the observed kinetics at two wavelengths could be related to specific skin pigments, the relative restored intensity, relative total remaining intensity and the local decay pattern eventually could serve as diagnostic parameters for optical discrimination of skin tissues.

Fig. 3. Skin autofluorescence intensity photo-bleaching under continuous 532 nm laser excitation (outer skin, power density ~85 mW/cm²). The curves are normalized to the initial fluorescence intensity.

Fig. 4. The changes of AF intensity under repeated 532 laser excitations of the outer skin area. The curves are normalized to the initial fluorescence intensity.
Fig. 5 illustrates AFPB time courses obtained in pre-irradiation experiments with three laser pairs. As the first step, the skin was pre-irradiated by 405 nm laser for 3 minutes. After one minute pause the same skin area was excited by 532 nm laser for AF photo-bleaching detection. The next measurement was taken at the neighboring spot of skin by another pair of lasers: 473 nm laser for pre-irradiation and 532 nm laser for AFPB recording. The third tested wavelength pair was the combination of 473 nm for pre-irradiation of another “fresh” skin area and 405 nm for AFPB recording from this spot. All laser output powers were adjusted to be approximately equal during the experiments.

Fig. 5. Left - the changes of 532 nm excited AF intensity after skin pre-irradiation by 405 nm and 473 nm lasers. Right - the changes of 405 nm excited AF intensity after pre-irradiation by 473 nm laser. Power density at each laser wavelength ~35 mW/cm². The curves are taken at the fluorescence band’s maxima wavelengths and are normalized to the initial (not pre-irradiated) fluorescence intensity.

Fig. 6. Temporal and spectral changes in the 473 nm excited skin autofluorescence signals, induced by 3-minute pre-irradiation using the same and two other lasers (405 nm and 532 nm). Left – the AFPB curves taken at 530 nm, right – the corresponding skin autofluorescence spectra.
Fig. 6. illustrates the skin AFPB effects observed at 473 nm laser excitation without and with pre-irradiation at the blue, violet and green wavelengths. One can see that the green 532 nm pre-irradiation does not influence much the fluorescence signals, but both 473 nm and 405 nm pre-irradiations cause significant signal changes. Again, the 473 nm pre-irradiation “cuts-off” the fast AFPB component, while 405 nm pre-irradiation mainly decreases the AF intensity without significant influence to the AFPB time course. The most pronounced spectral changes were observed after 405 nm pre-irradiation (Fig. 6, right). The blue-excited autofluorescence ratio, calculated as

$$I(I_o, \lambda) = \frac{\Delta I_{I_o}}{I_o} = \frac{I(\lambda)_{t=0} - I(\lambda)_{t=5 \text{ min}}}{I(\lambda)_{t=0}}$$

(1),

exhibit two peaks in the 540-590 nm spectral range, that coincide well with the two characteristic hemoglobin absorption peaks (Fig. 7). This observation may indicate to some role of hemoglobin in the skin AFPB process at the 473 nm excitation, and the 405 nm laser pre-irradiation probably increases this role.

Fig. 7. Relative loss of 473 nm excited autofluorescence intensity (calculated accordingly to (1)) after pre-irradiation of the same skin spot by 3 different laser lines, in comparison with the total hemoglobin absorption spectrum.

The spectral imaging setup was used to acquire fluorescence images like these shown in Fig. 8. Skin photo-memory effect in this case exhibits as outlines of the skin shield that was used to cover the pre-irradiation by another laser wavelength. The preliminary irradiated skin areas fluorescence with a lower intensity, as expected. By changing the wavelengths of the laser for pre-irradiation and the laser for AF image registration, the photo-memory effect was observed in all above mentioned combinations.
4. CONCLUSIONS

Results of the present study confirm photo-induced structural changes of healthy in-vivo human skin after cw visible laser irradiation at power densities of the range 35-85 mW/cm², i.e. well below the European skin safety limits. Autofluorescence photo-bleaching effects were observed at 532 nm, 473 nm and 405 nm laser excitations, and “photo-memory” signatures were recorded at all combinations of these wavelengths for pre-irradiation and fluorescence excitation. The obtained results may appear useful for better understanding of the mechanism(s) of skin autofluorescence photo-bleaching in further studies. It might open new possibilities for optical skin assessment by selection the most appropriate pre-irradiation/excitation wavelength combinations in order to reflect specific skin fluorophore responses.

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