The engraftment dynamics evaluation of skin grafts via aluminium phthalocyanine nanoparticles using spectroscopic methods

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The engraftment dynamics evaluation of skin grafts via aluminium phthalocyanine nanoparticles using spectroscopic methods

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Abstract. The research and comparison of spectroscopic methods for assessment of skin grafts engraftment/rejection after the back cross skin transplantation of laboratory mice were carried out. The spectral analysis was performed via inflammatory reaction evaluation of the studied area. The inflammatory reaction intensity correlates with the fluorescence intensity of the aluminum phthalocyanine nanoparticles. The photodynamic therapy was carried out to improve the skin engraftment and reduce the inflammatory reaction.

1. Introduction

The main problem of transplantology is inflammation of transplanted tissue that leads to serious complications of septic (microbial) or aseptic (autoimmune) nature. Today there are no non-invasive methods for assessing engraftment of grafts that are not time-consuming. The most well-known methods for vascular imaging used in clinic are MRI and CT. Unfortunately, these methods require contrast agents injection, have low resolution (0.5 mm), long scanning time and high cost [1]. One of the perspective method is laser-induced fluorescent diagnostics [2]. Usually wounds and burns are accompanied by inflammatory processes that affect to the fluorescent properties of tissue [3]. These properties depend on the fluorescence of normal tissues, microbial products in tissue and exogenous fluorophores that are used to accelerate wound healing [4].

One of the most perspective classes of nanophotosensitizers for inhibiting the inflammation are photosensitizers based on porphyrin [5] and nanoparticles of aluminium phthalocyanine (nAlPc), which have been studied intensively by various research groups [6-10]. We used nAlPc, the molecular nanocrystals capable of forming stable colloidal suspensions.

The main reason for the nAlPc use is the extremely low or nonexistent fluorescence of the nano form compared to high fluorescence intensity of the soluble form of AlPc (figure 1b). This fact is explained by the fluorescence quenching of nAlPc crystal structure [11]. But nAlPc begin to show fluorescent properties when entering the inflamed biological tissue. According to the authors [11] this is due to the presence of a large number of macrophages and pathogenic microflora in the inflamed tissue. nAlPc have several competing processes, such as dissolution and/or change of the particle surface structure while interacting with this microenvirenment. Now, the physics of this process are being studied, but the authors [12] have proposed the most likely hypothesis about the transition of

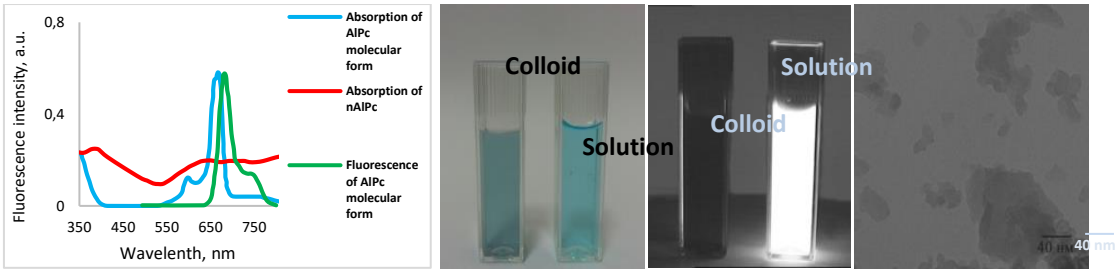
nanoparticle surface molecules from para-position to ortho-position with nanoparticles becoming fluorescent and photodynamically active.

The fluorescence lifetime is one of the important characteristics of fluorescence. According to the literature, the number of components and the fluorescence lifetime changes in the interaction with cells and depending on the pH. Thus, the presence of two fluorescence lifetimes were recorded at pH=2: 5 ns which corresponds to the molecular form in the solution and 1.5 ns which corresponds to the bound state of the phthalocyanine molecule. The study of the fluorescence lifetime of nAlPc in macrophages showed the presence of two components at 9 ns and 4.5 ns [13]. During our study, a change in the fluorescence decay kinetics was recorded, expressed in the appearance of additional fluorescence decay components.

2. Methodology

2.1. Nanophotosensitizer

nAlPc were used for the spectral analysis of skin healing grafts. The nAlPc colloidal solution was obtained by ultrasonic dispersion of large-dispersed crystals of aluminum phthalocyanine [14]. Thus, nAlPc with a hydrodynamic radius from 30 nm to 250 nm are possible to obtain via this method. Nanoparticles are plates with a diameter of 240±35 nm and a thickness of less than 50 nm (figure 1c).



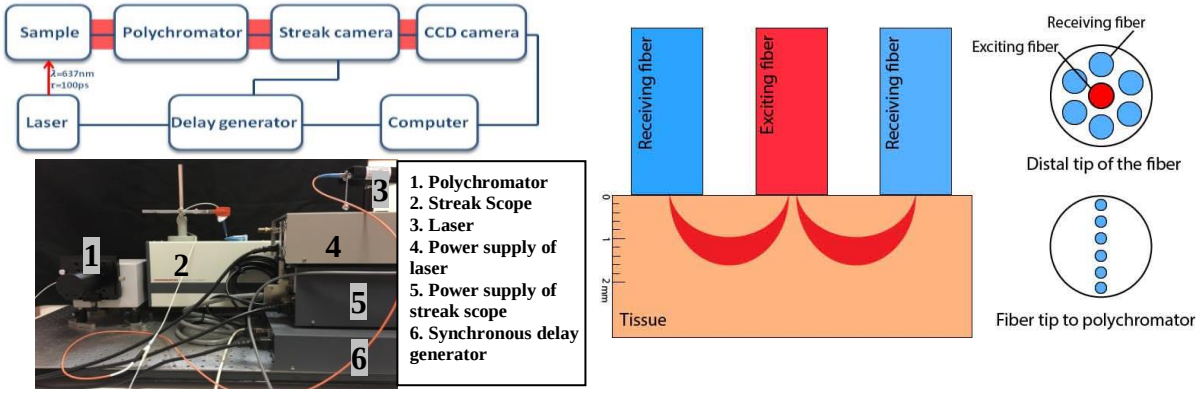
(a) (b) (c)

Figure 1. (a) absorption spectra of nAlPc colloidal solution and sulfated AlPc solution and fluorescence spectra of sulfated AlPc solution, (b) AlPc molecular solution and nAlPc colloidal solution in visible light and fluorescent image, (c) transmission electron microscopy image of nAlPc.

2.2. Experimental setup

Changes in the interstitial distribution of macrophages and other immune cells were evaluated via a streak-camera with picosecond time resolution (15 ps) by measuring the fluorescence lifetime of nAlPc. The streak-camera is paired to a fiber-optic spectrometer with 637 nm wavelength excitation and 65 ps pulse duration (figure 2).

The luminescence excited in the sample by the light from the laser source is collected via an optical fiber. A laser pulse is delivered to the sample through the central fiber and 6 peripheral fibers collect the backscattered light as well as the fluorescence. The central-symmetrical position of the fibers on the distal probe tip makes it possible to detect fluorescence regardless of the probe location to the sample (figure 2b). The luminescence and backscattered light are decomposed into the spectrum which then falls onto the photocathode at the streak scope input. The emitted electrons are accelerated by an electrical field parallel to the optical axis inside the streak scope. A perpendicular field is applied to the electrons for the duration of each scan. The electrons fall on the MCP (microchannel plate – analogue of the photoelectron multiplier with a 2d resolution), which amplifies the electron flow as a result of secondary electron emission (figure 2a) thus creating a flash on the phosphorescent screen. This flash is registered by a CCD camera and converted to a single photon event by the software. The mathematical processing of these events allows obtaining information on the fluorescence lifetime and the distribution of the number of photons between the components of the fluorescence decay registered by the streak-camera.



(a) (b)

Figure 2. (a) The flow chart of the experimental setup and the image of the experimental system, (b) the working principle of the fiber-optic probe consisting of 7 optical fibers.

2.3. Animal experimental design

The studies were carried out on the laboratory mice. Cross skin transplantation on the mouse back was performed similar to the study [4]. 10 µl of nAlPc with a 100 mg/kg concentration were added under the right skin graft (G500) while the left skin graft was used as control until the skin tissue was fully healed. In addition, skin tissues from mouse ears were used as "thin" skin grafts (G250). The thickness of the grafts were 500±50 µm for G500 and 250±30 µm for G250.

The photodynamic therapy was performed on day 18 after cross skin transplantation with 195 J/cm^2 dose with 500 mW/cm^2 power density and a laser source excitation wavelength of 668±10 nm in order to reduce the degree of inflammation in the area of the tissue to be healed. Fluorescence spectra were registered by the method of diffuse backscattered light from biological tissue using 633 nm laser source providing information about the state of the skin at depth. Measurements of nAlPc fluorescence lifetime and registration of nAlPc spectra were carried out on days 1;4;7;10;16;18;22;29 after cross skin transplantation.

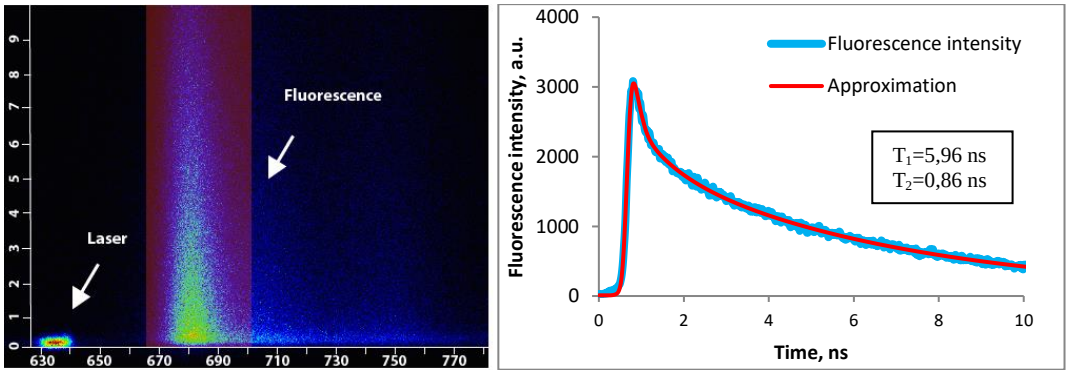
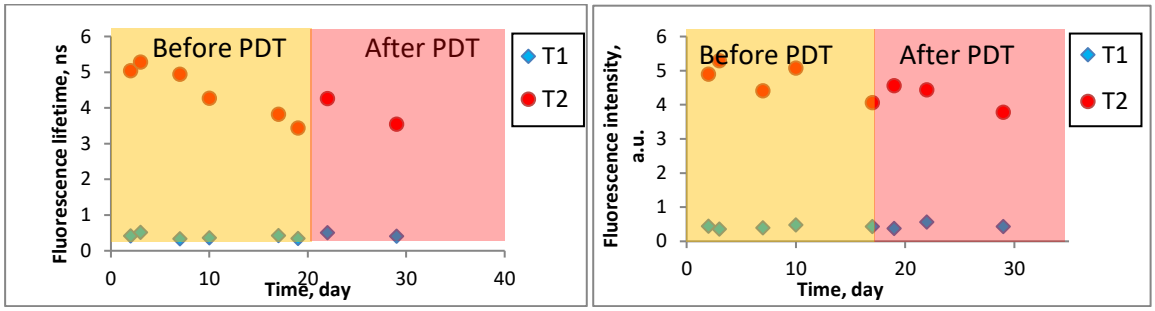
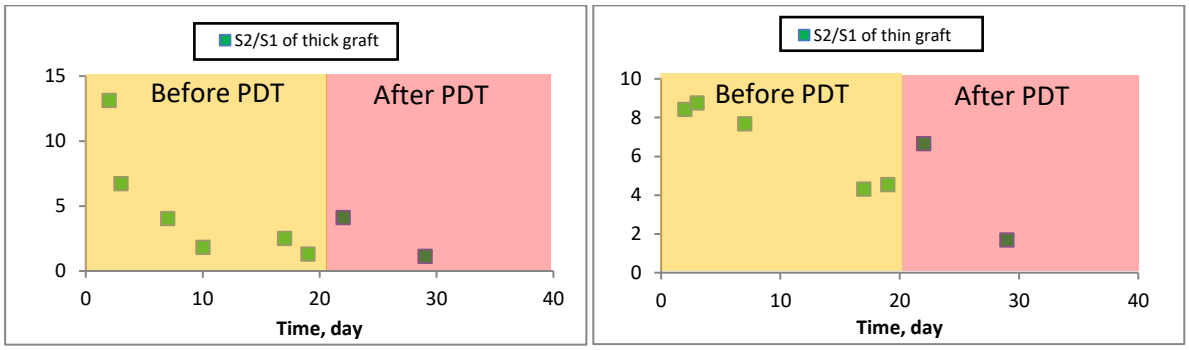


Figure 3. (a) the spectrum of nAlPc fluorescence decay under a G500 on day 7 after cross skin. transplantation, (b) the fluorescence decay after picosecond light pulse excitation.



(a) (b)

Figure 4. The dependence of nAlPc fluorescence lifetime on time (a) G500 (b) G250.



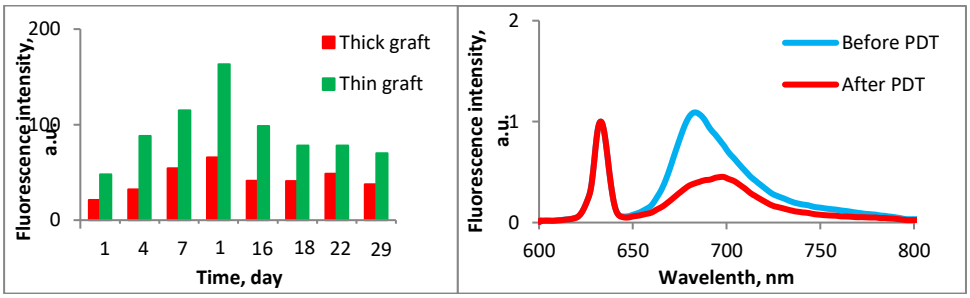
(a) (b)

Figure 5. The dependence of the long (S_2) and short (S_1) components photon number ratio of the nAlPc fluorescence lifetime on time (a) G500 (b) G250.

3. Results and discussions

In this work, the dynamics of the nAlPc fluorescence intensity under G500 and G250 grafts was obtained.

Two components of the nAlPc fluorescence lifetime under both grafts were recorded during the experiment. The nAlPc fluorescence lifetime itself varies slightly, but the contribution of each component to the total fluorescence varies significantly (figure 5). Particularly, the intensity ratio between long and short components changed from 13:1 to 1.5:1 during the experiment for G500 and from 9:1 to 2:1 for G250.



(a) (b)

Figure 6. (a) Histogram of the fluorescence intensity of G500 and G250 grafts with nAlPc, (b) fluorescence intensity of G500 before and after PDT.

Figure 6a shows the fluorescence index calculated as a ratio between the nAlPc fluorescence and the backscattered laser light for G500 and G250 according to the equation:

$$k_{fl} = \frac{\int_{660}^{725} I(\lambda)\mathrm{d}\lambda}{\int_{623}^{640} I(\lambda)\mathrm{d}\lambda} \quad (1)$$

The nAlPc fluorescence intensity increases within 10 days after cross skin transplantation of both grafts. Furthermore, the fluorescence intensity in the graft tissue decreases which indicates the successful graft engraftment. On average, the fluorescence intensity of G250 with nAlPc is lower due to the absorption of laser radiation by blood cells. Consequently, the thickness of the graft tissue greatly affects the obtained results in this measurement geometry. Meanwhile the changes of the nAlPc fluorescence intensity are similar in both grafts. PDT was performed on day 18 after cross skin transplantation with the aim to improve the engraftment speed of the skin graft and nAlPc photobleaching (figure 6b). There is a noticeable decrease of the nAlPc fluorescence intensity in G250 after PDT. The spectral curve change is due to a different environment inside the graft after PDT, which affected the change in the nAlPc fluorescence lifetime.

The successful engraftment of skin grafts took place in the experiment, and while there is a strong purulent inflammation under the control graft, there was no pus under the graft with nAlPc.

4. Conclusion

The engraftment of skin grafts was evaluated via nAlPc during the experiment. By changing the fluorescence intensity, which correlates with the inflammatory reaction, the degree of graft engraftment can be assessed.

An interesting new effect was demonstrated that the ratio of the long to short components of the nAlPc fluorescence decay is significantly different after PDT. Presumably, this characterizes the changes in nAlPc local microenvironment, which is determined primarily by the type of immune-competent cells involved in the inflammation. This method can be useful for assessment of graft engraftment and allows preventing the rejection.

Acknowledgements

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