Ytterbium porphyrins complexes for NIR-luminescent diagnostics and magneto-luminescent theranostics of tumours

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Abstract. Promising ytterbium porphyrins complexes (YbPC) and nanocomposites on its base for NIR-luminescent diagnostics and magneto-luminescent cancer theranostics are presented. The considered complexes are very promising substances for the cancer luminescent diagnostics (LD) in the NIR spectrum region (800–1100 nm). For LD of malignant tumors of the skin and mucous membranes, a pharmaceutical composition of the «Fluroscan» type was developed. It has been established that YbPC can also be used as one of the main components of the nanocomposites created for cancer theranostics, including magneto-luminescent theranostics (MLT). For the purposes of MLT, a 3-component nanocomposite was developed, consisting of a polymer matrix (Lexan type), a diagnostic substance based on the ytterbium complex of hematoporphyrin IX tetramethyl ester, and a therapeutic FeO$_x$ component.

1. Introduction

There are two approaches in neoplasms luminescent diagnostics (LD): using special exogenous fluorescent markers administered into the body, and without using them, when tumors are diagnosed by fluorescence of endogenous (inherent to every living organism) porphyrins that accumulate in tumor and other rapidly proliferating biological tissues. For primary LD and for LD accompanying cancer photodynamic therapy (PDT), therapeutic FS are usually used.

Photogem, Photofrin-2 (first-generation PS drugs), Foscan, Fotosens, Photoditazin,, Alasens, based on 5-ALA – 5-aminolevulinic acid, protoporphyrin IX (PP IX) and others (second-generation drugs) are used for cancer PDT, as well as for accompanying LD. Fluorescence peaks are due to the predominant PS accumulation in a tumor. However, the intrinsic tissues fluorescence (from endogenous porphyrins and other chromophores inherent in biological tissues) also contributes to this spectral range, which must be taken into account when assessing the PS concentration in tissues.

Thus, it is obvious that the perspective of using therapeutic PS for effective early cancer diagnostics is rather doubtful, and a use of only the spectral range (600–750 nm) in the process of tumors early diagnosis, in our opinion, is not optimal.
Many researchers claim that the NIR range is the most promising for biomedical diagnostic studies because of the greater depth of photon penetration through biological tissue and minimal autofluorescence in this spectrum range [1–3]. Recently, a number of studies have appeared [4, 5], in which, for various biomedical applications, including the neoplasms diagnostics, low-toxic photosensitizers based on some lanthanide porphyrins complexes that luminesce in the 800–1100 nm NIR spectrum region (NIR-luminescence), in the so-called “transparency window of biological tissues”. In this range the background endogenous porphyrins luminescence is practically absent. Among FS ytterbium porphyrins complexes are the most promising substances for cancer LD. These compounds are characterized by a high intensity luminescence signal in the 900–1100 NIR spectrum region, have a high extinction coefficient (1.5 × 105 M⁻¹ cm⁻¹) and an increased luminescence lifetime of 5–10 μs [6].

It was shown [6] that the insertion of ytterbium ion into the porphyrin matrix center, as follows from theoretical constructions, should lead to an abrupt decrease in photochemical activity, while keeping the characteristic for most porphyrins tropism to malignant tumors. As a result, the excitation of the porphyrin matrix under the influence of external light radiation is not transmitted to oxygen, but is intercepted by the Yb³⁺ ion, thereby sharply reducing the singlet oxygen generation sensitized by a porphyrin. Herewith luminescence is observed due to transitions of 4f-electrons of the Yb³⁺ ion: ²F₇/₂ → ²F₅/₂.

A new direction in modern nanobiotechnology is the development of multifunctional composites for cancer theranostics, which combine diagnostic and therapeutic properties in one nanoparticle [7, 8]. It was previously shown [9] that YbPC can also be used as one of the main components of the nanocomposites created for cancer theranostics. So, together with co-authors, we developed a theranostics method using a multifunctional nanocomposite that provides NIR-luminescent diagnostics based on YbPC in combination with plasmon resonance photothermotherapy [9].

In this work, we propose the use of some perspective YbPC for purposes of NIR-luminescent diagnostics of skin neoplasms and mucous membranes, as well as for theranostics of tumors based on NIR-luminescent diagnostics in combination with radiofrequency magnetic thermotherapy – magneto-luminescent theranostics (MLT).

2. Results and discussions

2.1. The development of the neoplasms early NIR-luminescent diagnostics method based on YbPC

It was previously established that one of the most promising compounds for the neoplasms NIR-luminescent diagnostics is the Yb/mol-complex of 2,4-di(α-methoxyethyl) deuteroporphyrin IX (Yb-DMDP) [10]. The total luminescence quantum yield Φₜot of ytterbium ions in the studied complexes was calculated using a solution of Zn-tetraphenylporphyrin in ethanol as a reference, the quantum yield of which is 0.03 [11]. The calculated luminescence quantum yield of the ytterbium ion was ~ 1%. It was also found that the average luminescence lifetime for the synthesized ytterbium complexes is 5–10 μs.

For LD malignant neoplasms of the skin and mucous membranes, the “Fluroscan” pharmaceutical composition was developed, consisting of the dipotassium salt of Yb-DMDP, luminescent in the NIR spectral region (900–1100 nm) and various gels (cremophor, tizol) using DMSO, glucosamine and glycerol, which provide good permeability to the skin and mucous membranes [12]. Examples of clinical researches results are shown in figure 1. The study was carried out on 30 patients. Most patients had multiple and combined skin lesions. Of these: basal cell without ulceration – 18, basal cell with ulceration – 12, seborrheic keratosis – 28, patients with actinic keratosis – 7. The excitation wavelength is ~ 405 nm, the wavelength of the recorded luminescence is 900–1100 nm. In this case (figure 1), we studied the integrated luminescence intensity (in the spectral range 900–1100 nm) and, accordingly, the YbPC accumulation in various elements of neoplasms and healthy skin areas. Using the graph, one can evaluate the value of the luminescent diagnostic contrast index (LDCI):

\[
\text{LDCI} \left( \frac{I_{\text{pos},4}}{I_{\text{pos},6}} \right) \sim 1.3 \text{ arb. units} / 0.2 \text{ arb. units} = 6.5.
\]
It should be noted that for all patients, during the treatment process, a gradual decrease in the YbPC luminescence intensity to values characteristic of healthy tissues was observed.

An interesting fact is that visually unchanged skin areas had an increased luminescence in places where fresh inflammatory elements appears later (after 3–4 days).

Figure 1. Patient M., 65 years old. Basal cell skin cancer (superficial form): 1, 2, 3 – areas of tumor growth without ulceration; 4 – ulceration along the edge of a lesion, 5 – skin not treated with the gel (outwardly healthy), 6 – unchanged skin treated with the gel.

2.2. The development of nanocomposites based on the Lexan-polymer matrix, ytterbium porphyrins complexes and magnetic nanoparticles for MLT

It should be noted that for today methods using magnetic nanoparticles (MNP) and heating radio frequency electromagnetic fields (EMF) are quite developed and continue to improve. Among them is magnetic hyperthermia (MHT), while the frequency of the electromagnetic field is 200–1000 kHz. Recently, non-thermal mechanisms of magnetic theranostics (the mechanism of magneto-mechanical actuation) using non-heating low-frequency electromagnetic fields (1–1000 Hz) have also been developed [13].

The MNP use allows to locally increase the exposure intensity and reduce the frequency and magnitude of the required induction of a variable EMF, and therefore, reduce the chance of undesirable side effects from the field [14]. In most biomedical applications, magnetite Fe₃O₄ is used as a material of a magnetic core (Jₛ ≈ 80 A·m²/kg – magnetite saturation magnetization), which has significantly lower toxicity than pure magnetic metals and many magnetic alloys.

For the theranostics purpose a ternary nanocomposition was developed, consisting of a polymer matrix, a diagnostic substance based on YbPC and a therapeutic composition component – Fe₃O₄. The polycarbonate bisphenolic polymer Lexan was chosen as the polymer base (Lexan polymer matrix – LPM). In order to give hydrophilicity to the particles surface and, therefore, a less aggregation and longer circulation in the body, the nonionic detergent Triton X-100 is also added into the organic phase. During the particles formation, the detergent hydrophobic part was embedded in the particle and the hydrophilic (polyethylene glycol) remained on the surface. For better stabilization of the ytterbium complex, an additional complexing agent, trioctylphosphine oxide (TOPO), was used.

The acetylacetonate ytterbium complex of hematoporphyrin IX tetramethyl ester (Yb-TME HP) was obtained in accordance with the method described in [10]. The Lexan – manufactured by General Electric Plastics, USA; bovine serum albumin (BSA), tetrahydrofuran (THF), TOPO, Triton X-100 – manufactured by Sigma-Aldrich, USA. The TOPO inclusion in the YbPC was carried out in THF solution for 15 min before the nanoparticles synthesis. The synthesis of Lexane nanoparticles loaded with Yb-TME HP was carried out by the analogy with the method proposed in [15], in our modification leading to the receiving of larger diameter particles [12].

Fe₃O₄ iron oxide nanoparticles were obtained using the method of metal wire electric explosion by means of high voltage electric pulses (30 kV, 1 Hz). A detailed description of the method and equipment is given in [16, 17]. Nanoparticles containing the iron oxides core and the polymer shell including the
YbPC (structure: LPM + YbPC + FeO\(_x\)) were synthesized according to the following procedure. To the voiced aqueous suspension of FeO\(_x\), the pre-mixed solutions of the Lexan, Yb-TME HP, TOPO and Triton X-100 in THF (in the above proportions) were added, sonication was continued for 3 min, after which THF was evaporated at 50 °C, the volume was adjusted to the original (5 ml) and then the analyzes were carried out. The structural diagram of the synthesized nanocomposite LPM + YbPC + FeO\(_x\) for MLT is presented in figure 2.

![Polymer shell](image)

**Figure 2.** Scheme of the LPM + YbPC + FeO\(_x\) nanocomposite, where PS is Yb-TME HP.

**Figure 3.** The nanoparticles emission spectra in the NIR region (\(\lambda_{\text{ex}} \sim 532\) nm): 1 – LMP + Yb-TME HP composite, 2 – LMP + Yb-TME HP + FeO\(_x\) composite.

The carried out assessment of the synthesized nanoparticles size distribution showed that with an increase in the polymer concentration in the reaction volume particles of a larger diameter are formed. The nanoparticles size in the suspension was determined by the dynamic light scattering method on a laser correlation spectrometer of “Kurs-3”-type, which allows to carry out measurements in the range from 0.5 nm to 104 nm.

In figure 3 typical examples of the emission spectra of synthesized nanoparticles aqueous suspensions are shown. Concentrations of ingredients used in the synthesis of the composite LPPM+ YbCP + FeO\(_x\) are presented in the table 1.

**Table 1.** Suspension components concentrations used in the synthesis of nanoparticles containing a FeO\(_x\) core and Yb-TME HP in a Lexan polymer shell.

| Nanoparticles series | FeO\(_x\), mcg/ml | Yb-TME HP, mol/l | TOPO, mol/l | Lexan, mcg/ml | Triton X-100, % |
|----------------------|-------------------|-----------------|-------------|--------------|----------------|
| 10                   | -                 |                 | 12x10\(^{-6}\) | 180          |                |
| 12                   | 80                |                 | 12x10\(^{-6}\) | 180          |                |
| 13                   | 80                | 4x10\(^{-6}\)   | 24x10\(^{-6}\) | 180          | 0.035          |
| 14                   | 80                |                 | -            | 180          |                |
| 15                   | 80                |                 | 24x10\(^{-6}\) | 270          |                |
| 16                   | -                 |                 | 24x10\(^{-6}\) | 270          |                |

Note: used amount of Lexan corresponds to calculated thickness of a polymer shell around 15.2 nm FeO\(_x\) core, which is equal to ~ 21 nm (series 12–14) and ~ 30 nm (series 15).

The nanoparticles emission spectra in the 800–1060 nm were obtained using the developed at the Kotel'nikov’ Institute of Radio Engineering and Electronics RAS measuring stroboscopic test-bench with excitation in the visible spectrum range [18].
Presented in figure 3 the nanoparticles emission spectrum in the NIR-range confirms that it retains the typical character for Yb$^{3+}$ ion luminescence: the presence of the most intense band at 980 nm and the appearance of additional bands in the 925 nm and 1010 nm regions due to intramultiplet transitions. From the presented data one can see that the Yb$^{3+}$ ion luminescence intensity $I$ and lifetime $\tau$ increase with the TOPO content increase in the polymer matrix, and to obtain the highest values it requires ~ 2-fold excess of TOPO.

It is to be noted that the FeO$_x$ nucleus incorporation into the nanoparticles leads to an approximately 2-fold decrease in the Yb$^{3+}$-luminescence intensity in the synthesized nanoparticle variants. In the TOPO absence luminescence is practically not observed. The main luminescence fraction falls on the finest dispersed (supernatant) fraction, which indicates that a coarse suspension containing “large” FeO$_x$ nuclei practically does not luminesce. The data presented indicate that in order to obtain better luminescent nanoparticles with an iron oxide core containing YbPC, it is necessary to use a more monodisperse initial FeO$_x$ suspension with a diameter of no more than 10 nm.

We also studied the nanocomposite biodistribution in the animals organs and tissues (female mice of the Bulb / c line with Ehrlich's inoculated carcinoma) at certain time intervals from the moment of its administration. The studies were carried out on the testing laser-fiber fluorimeter (LFF) developed in the Kotelnikov’ Institute of Radio Engineering and Electronics RAS, Fryazino branch. Presented data show a significant selectivity of the nanocomposite accumulation in the tumor 12 hours after intravenous administration. This result can be primarily explained by the size effect (the size of the composite nanoparticles does not exceed 200 nm). As was shown in [19], nanoparticles use defects in the tumor microvascular system, which leads to the implementation of the so-called effect of enhance permeability and retention (EPR). The synthesized nanoparticles size (100–200 nm) suggests the possibility of their preferred accumulation in the tumor tissue due to the penetration through tumor blood vessels endothelial defects, as well as due to the natural tumor tropy of the porphyrin complex part.

3. Conclusions

It was noted that the considered complex Yb-DMDP is very promising substance for the cancer luminescent diagnostics (LD) in the NIR spectrum region (800–1100 nm). For LD malignant neoplasms of the skin and mucous membranes, the “Fluroscan” pharmaceutical composition was developed, consisting of the dipotassium salt of Yb-DMDP, luminescent in the NIR spectral region (900–1100 nm) and various gels. Note that the difference in luminescence levels between healthy skin and tumor foci varies significantly. In the future, the LD-technique on the base of "Fluroscan" gel can be applied for differential diagnostics of skin cancer, revealing hidden foci of tumor growth and monitoring the therapy effectiveness.

The synthesized structure of the LPM + YbPC + FeO$_x$ type can be in demand for MLT. The incorporation of the FeO$_x$ core into nanoparticles allows to be carry out a controlled local high-frequency hyperthermia of tissues that have accumulated the nanocomposite, although it leads to a slight decrease in its diagnostic potential (to an approximately 2-fold decrease in the intensity of YbPC luminescence). However, this disadvantage can be compensated by a slight increase in the therapeutic dose of the nanocomposite.

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