

Sol-gel Coated Fiberoptic Applicator for Photodynamic Medicine – Optical and AFM Characterization

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The application of spectroscopic study, microscopic and AFM imaging for examination of fiberoptic applicators is presented. The potential carriers of photoactive agents for photodynamic medicine in form of sol-gel coatings of fiberoptic applicators, are proposed. Optical and morphological properties of the proposed sol-gel coatings doped with photosensitizer Photolon, are characterized. The influence of pH and oxygen changes on entrapped Photolon properties, was examined, as well. The morphology of the applicator coating was examined by using atomic force microscopy. The light distribution from an applicator was studied by means of computer aided image analysis.

K e y w o r d s: photosensitizer, Photolon, biocarrier, fiberoptic applicator, PDT

1. Introduction

Precisely engineered materials for novel therapeutic and diagnostic modalities are recently widely examined. There is a growing interest in nanotechnology and nanomaterials that may be exploited among others for medical drugs carriers. The most common nanotechnology platforms include polymeric nanoparticles, nanoshells, micelles, liposomes, dendrimers, nucleic acid based nanostructures, engineered viral nanoparticles, magnetic nanoparticles, silicon oxide nanoparticles, and quantum dots [1–5].

The main goal of our work was to design and characterize a biocarrier of photosensitizer for photodynamic medicine in form of sol-gel coating on fiberoptic

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applicator. A fiberoptic applicator is proposed that may be applied in interstitial photodynamic medicine. The applicator at the distal part is covered by sol-gel coating with entrapped photosensitizer, whereas the sol-gel coating plays a role of carrier of an entrapped chromophore. The influence of pH changes and oxygen environment on the entrapped photosensitizer was examined. Chlorin derivative – Photolon was used as the photosensitizer. In organic chemistry, chlorin is a large heterocyclic aromatic molecule consisting of 3 pyrroles and one reduced pyrrole ring, all coupled by methine linkages. Because of their strong photosensitivity, one-photon-based photosensitizers as chlorines are widely used as photosensitizing agents in experimental and clinical PDT therapies [6, 7]. The dye is approved for cancer treatment in some countries [8]. In the last decade, some non-oncologic applications were reported, as well [9].

In photodynamic diagnosis (PDD) luminescence properties are extremely important. It is often observed that luminescence signal decreases with the time, even without any quencher (photobleaching). The luminescence intensity depends on dye concentration and power of the excitation source [10, 11].

Nanotechnology has a potential to improve cancer diagnosis and therapy. Advances in photosensitizers engineering and materials science have contributed to novel nanoscale solutions that may be beneficial for cancer patients. Several therapeutic nanocarriers for clinical use, have been examined [12, 13]. Some nanocarriers incorporate molecules to selectively bind and target cancer cells. Some nanocarriers and specific molecules for selective tumor targeting are described, as well [14].

Polymeric nanoparticles designed for controlled release systems can encapsulate drugs and release them in a controlled manner through surface or bulk erosion of the particles, diffusion of the drug through the polymer matrix, or swelling followed by diffusion. Alternatively, drug release can be triggered by the environment and external factors such as changes of pH, temperature, oxygen concentration, or the presence of an analyte such as e.g. glucose. Liposome-based drug delivery systems are built of nanoparticles through the assembly of amphiphilic bi-layer membrane composed of natural or synthetic lipids [15, 16].

Optimal delivery of pharmaceutical, therapeutic, and diagnostic agents is at the forefront of many projects in nanomedicine. Bionanocarriers may help to overcome the limitations of ordinary drugs delivery methods. Some of these systems have been extensively investigated for cancer therapy and diagnosis applications [17–20].

Various fluorochromes such as, e.g. cyanine dyes or porphyrins have been described, demonstrating variable stabilities, quantum efficiencies, and ease of synthesis. Porphyrins may be used for both, photodynamic diagnosis, as well as therapy and there exists a number of relevant publications [21]. The less known are chlorine based agents, e.g. Photolon, which may be used in photodynamic diagnostics [22], and exhibits a potential anti-tumor activity, as well [23]. The Photolon's properties may be modified due to the binding to PVP (polyvinylpyrrolidone) matrix [24]. Photolon shows strong absorption in the short wavelengths region (at ~400 nm) and

fluorescence maxima in the long wavelengths region (at 663 and 668 nm respectively), high fluorescence quantum yield (93%) and high quantum yield of singlet oxygen (65%) [22]. It was demonstrated that this agent may find an application for fluorescence diagnosis of malignant tumors [25]. The fluorescence imaging of various malignant tissues (in skin, heart, lung, gall bladder, liver, spleen, kidney and gastrointestinal tract) 1 h after intravenous administration was presented by Chin et al. [26].

Atomic force microscopy (AFM) can be used for high resolution imaging of objects in various environments: air, liquid or vacuum. Many biological systems, especially isolated macromolecules (mostly nucleic acids, polysaccharides and proteins), as well as cells, tissues and biomaterials, may be examined by AFM [27, 28]. In the current paper, nanostructural characterization was focused on the AFM examination of photosensitizer entrapped in the sol-gel carrier in form of 8-layered coating deposited on fiberoptic applicator. Fiberoptic applicators may initiate photodynamic reaction *in situ* and may find an application in minimal invasive treatment of interstitial tumors. Therefore, it is important to examine the influence of environment (e.g. pH, oxygen) on applicator performance.

2. Materials and Methods

2.1. Synthesis of Silica-based Sol

The sol-gel coatings were produced from sol prepared from the silicate precursor TEOS (tetraethyl orthosilicate) dissolved in ethanol with molar ratio 1:20. Next, hydrochloric acid (37%) was added as a catalyst to ensure an acid hydrolysis (pH ≈ 2). The mixture was stirred for 30 minutes at room temperature using a magnetic stirrer (speed 400 rot/min).

2.2. Immobilization of Photosensitizer in Silica-based Sol

Photolon:(18-carboxy-20-(carboxymethyl)-8-ethenyl-13-ethyl-2,3-dihydro-3,7,12,17-tetramethyl-21H, 23H-porphin-2-propionic acid) (Belmedpreparaty, Belarus) was used as a photosensitizer. 0.1% solution of the photosensitizer was prepared by dissolving Photolon in ethanol. The solution of Photolon was added to 1 ml freshly prepared sol. 20 µl of surfactant Triton X-100 (Aldrich) was added to 1 ml of sol material to lower the surface tension of a liquid.

2.3. Fiberoptic Applicator with Sol-gel Based Coating with Entrapped Photolon

Hard Clad Silica (HCS) fibers of low OH (CFO 1493-12), cladding TECS™ (φ₂ = 430 µm) and fused silica core (φ₁ = 400 µm) from Laser Components were used for the applicator preparation. The external jacket was mechanically removed

from one end on a distance of 2 cm. Afterwards, the fiber clad was removed by a hot torch. The residuals were removed with linen cloth and the bar fiber was cleaned with ethanol. The fiberoptic core was covered by silica sol doped with photosensitizer by dip-coating method, so thus to form the sol-gel coated applicator. The final coating was composed of 8 sol-gel layers.

2.4. Atomic Force Microscopy Examination

The AFM images were acquired in the tapping mode using Nanoscope IIIa scanning probe microscope with the Extender Module (Bruker) in the dynamic modus. An active vibration isolation platform, Halyonics, Mod. 1, was applied. Olympus etched silicon cantilevers were used with a typical resonance frequency in the range of 200–400 kHz and a spring constant of 42 N/m. The set-point amplitude of the cantilever was maintained by the feedback circuitry to 80% of the free oscillation amplitude of the cantilever. All samples were measured at room temperature in air. The sample was first adjusted with an optical light microscope (Nanoscope, Optical Viewing System). The distribution of photosensitizer molecules on the coating surface was analyzed.

2.5. Recording of Luminescence Spectra

Luminescence spectra were recorded by using of AvaSpec-3648 (Avantes) spectrophotometer. The photoexcitation set-up is schematically presented in Fig. 1. The system consists of: light source, measurement chamber, fiber holder, spectrophotometer and computer for data processing. Semiconductor laser (TOP–GaN, Poland) emitting 415 nm wavelength was used as an excitation source. Luminescence spectra of Photolon entrapped in the silica sol-gel coating were measured for the applicators exposed to pH and oxygen changes. The spectra were recorded for the applicator placed in the chamber and exposed to basic or acidic steam. In the experiment, the fiberoptic applicator was placed into a quartz chamber through hole in the chamber cup. Basic environment (pH=10) was achieved by adding to the chamber 1 ml of NaOH solution (2 mol/dm³). Acidic environment (pH=4) was achieved by adding to the chamber 1 ml of HCl solution (5 mol/dm³).

The influence of oxygen on the entrapped photosensitizer was examined for the oxygen constant flow of 5ml/min. Through the second pinhole an elastic pipe connected to the reducer with oxygenic demijohn O₂ was placed. The reducer was set to a constant flow of gas. Luminescence spectra were recorded over a time of 1, 3, 5 and 10 minutes after insertion of the applicator into the container.

To determine the spatial light distribution from the applicator light distribution was recorded by means of a CCD camera for two different wavelengths (red and blue diode: 660 nm and 430 nm, correspondingly). The image processing analysis in MatLab environment was performed.

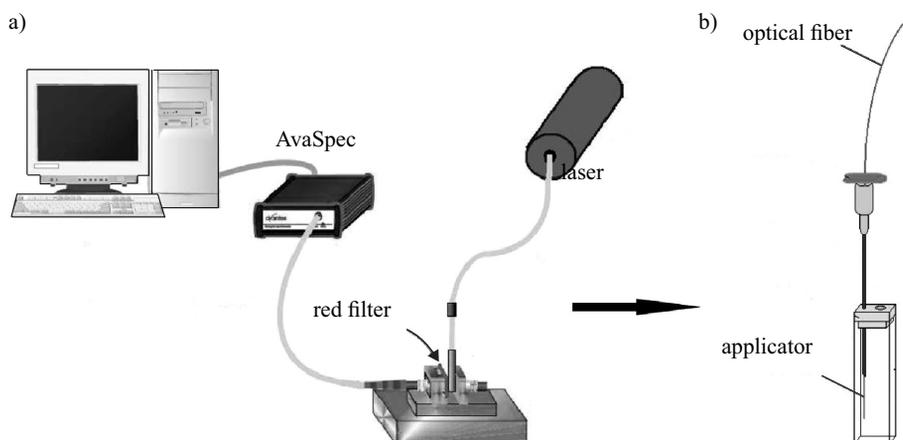


Fig. 1. Luminescence set up; a) luminescence set-up with the excitation source: semiconductor laser emitting 415 nm wavelength, b) fiberoptic applicator inserted into the measurement chamber

3. Results and Discussion

An exemplary microscopic image of the applicator recorded by optical microscope Nikon ECLIPSE E200 in reflection mode is shown in Fig. 2 (red light (Fig. 2a) and blue light (Fig. 2b)). The discrete spatial light intensities distributions (gray scale from 0 to 255) are presented on Fig. 2c and 2d. The distributions were obtained by using the contour function in Matlab. The achieved results demonstrate that the more effective light distribution appears for the red light than for the blue light propagating from the fiber. In the second case, the spatial light distribution is limited only to the space region located near to the fiber surface.

The spatial light distribution of the light emitted from the applicator is an important factor that may influence the ability to interact with tissue and initiate photodynamic reaction.

The surface morphology of silica sol-gel carrier was characterized by atomic force microscopy. The AFM images of doped and undoped sol-gel layers revealed that the coatings are smooth and homogeneous (see Fig. 3).

The measured roughness profiles are in the range of 0.5 to 2.0 nm. Photolon molecules are visualized, as well. One may see that molecules are non-uniformly distributed in the matrix and visible on the top of the silica sol-gel film (Fig. 3b). It may indicate that the photosensitizer will be able to interact with tissue.

The luminescence spectra were measured for the applicators exposed to pH changes (Fig. 4), as well as to oxygen flow (Fig. 5). The luminescence spectra of the entrapped Photolon were recorded for three various pH: pH=7 (Fig. 4a), pH=4 (Fig. 4b) and pH=10 (Fig. 4c). A significant influence of pH was observed for the applicator placed in the acid environment (Fig. 4c). The luminescence intensity in-

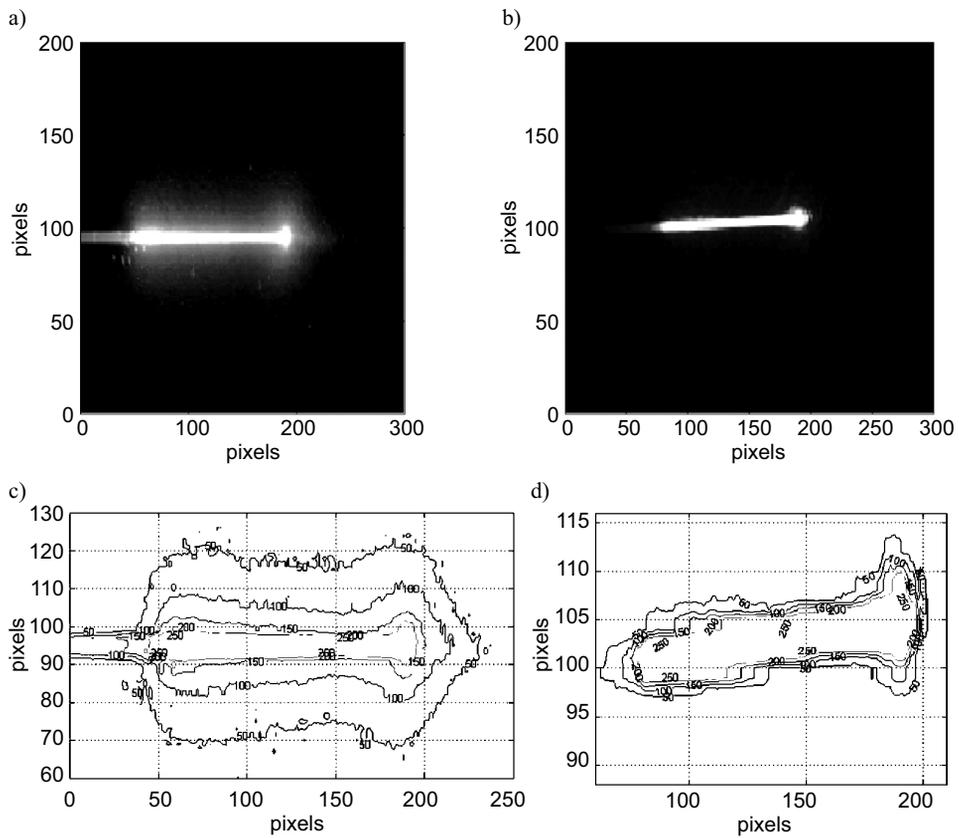


Fig. 2. Spatial light distribution from the applicator: a, c) for red light and b, d) for blue light. Recorded images (a, b) and light distribution discrete maps (c, d)

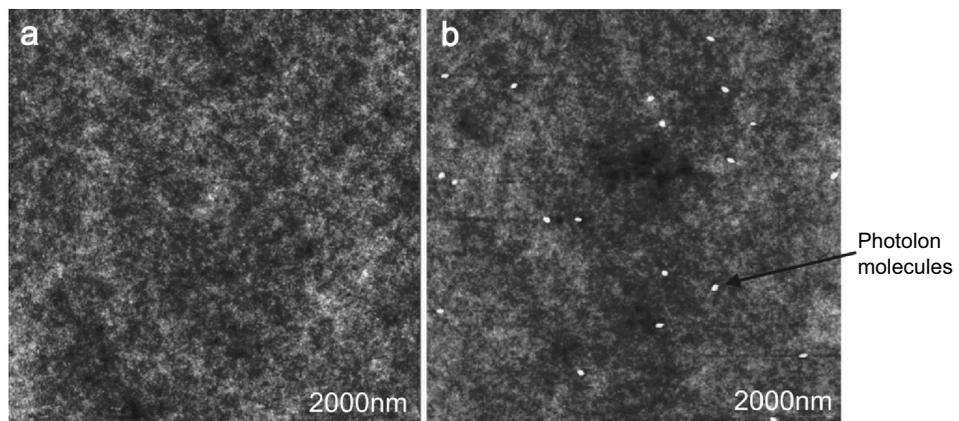


Fig. 3. AFM images of a) undoped silica sol-gel carrier on mica, b) silica sol-gel coating doped with Photolon on mica (white spots denote Photolon molecules)

creased comparing to the measurement in the neutral environment (Fig. 4a). The shift of signal maximum from 675 nm to 670 nm was also observed for pH=4. Whereas, the luminescence intensity for the applicator placed in the basic environment decreased in compare to the neutral one (Fig. 4b). Additionally, for all three environments the decrease of luminescence intensity was observed over a time. However, in the case of acid environment the luminescence is still detectable even after 30 minutes of irradiation, whereas in the neutral environment the luminescence signal decreased completely after 15 minutes. In the basic environment the quenching of luminescence signal was observed after 10 minutes.

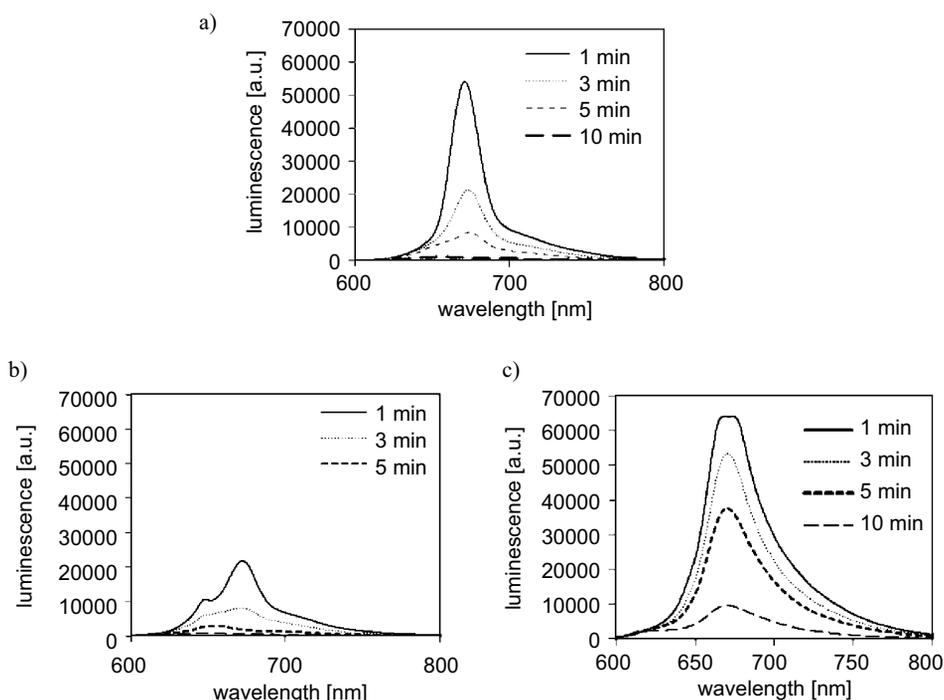


Fig. 4. Influence of pH on luminescence spectra of entrapped Photolon a) pH=7, b) pH=10, c) pH=4. Spectra recorded 1, 3, 5 and 10 minutes after insertion of the applicator into the container

The influence of oxygen was characterized over a time of 10 minutes, for the constant oxygen flow 5 ml/min. The recorded spectra are presented in Fig. 5. The luminescence intensity of the entrapped Photolon measured for the applicator placed in the chamber with oxygen gas decreased in comparison to the applicator placed in air. Oxygen concentration higher than in air - resulted in the quenching of luminescence. The effect of the luminescence quenching caused by high oxygen concentration and the photosensitizer bleaching, were reported elsewhere [29–32].

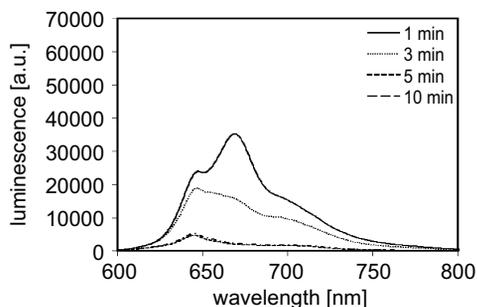


Fig. 5. Influence of oxygen flow 5 ml/min on luminescence spectra of entrapped Photolon. Measurements recorded after 1, 3, 5 and 10 minutes of exposure to oxygen. Reference luminescence spectra recorded in the air are presented in Fig. 4a

4. Conclusions

A fiberoptic sol-gel coated applicator was designed, whereas the coating may serve as a carrier of photoactive dye.

The surface morphology of the sol-gel coating was characterized by using atomic force microscopy. It was shown that the surface is smooth and homogenous, although the distribution of the entrapped photosensitizer is non-uniform. Photolon molecules were observed on the top of the surface layer.

The luminescence signals of the entrapped photosensitizer were measured. The influences of pH and oxygen flow on the optical properties of photoactive dye entrapped in the sol-gel coating were examined. We observed a significant increase of the luminescence intensity in low pH, whereas in the neutral and basic environments the luminescence intensity decreased.

The oxygen flow quenches the fluorescence signal of photosensitizer, what, from the other hand, may be exploited in designing of fiberoptic oxygen sensor. It may be also possible to monitor the changes of pH and oxygen in the tissue during the photodynamic treatment.

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